

**CHARACTERISATION OF PATHOGENS ASSOCIATED WITH TRUNK DISEASES
OF GRAPEVINES**

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

Signature:

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SUMMARY

In an attempt to combat some of the pathogens that are associated with trunk diseases and disorders of grapevines, research in this thesis focused on the taxonomy and pathological aspects of *Coniella/Pilidiella*, *Botryosphaeria* and *Phomopsis* spp.

Previously, conidial pigmentation was used to separate *Pilidiella* from *Coniella*. Recently, however, the two genera have been regarded as synonymous, with the older name, *Coniella*, having priority. The most important species in the *Coniella/Pilidiella* complex of grapevines is *C. diplodiella* (Speg.) Petr. & Syd., the causal organism of white rot of grapevines. Previous studies found it difficult to distinguish between *C. diplodiella* and *C. fragariae* (Oudem.) B. Sutton, which is known to occur in soil and caused leaf diseases of *Fragaria* and *Eucalyptus*. Both these species have previously been reported from South Africa. None of the reports on *C. diplodiella* could be scientifically substantiated; therefore it is still a quarantine organism. However, this status has been questioned. Based on sequence analyses of the internal transcribed spacer region (ITS 1, ITS 2), 5.8S gene, large subunit (LSU) and elongation factor 1- α gene (EF 1- α) from the type species of *Pilidiella* and *Coniella*, *Coniella* was separated from *Pilidiella*, with the majority of taxa residing in *Pilidiella*. *Pilidiella* is characterised by species with hyaline to pale brown conidia (avg. length: width >1.5), with *Coniella* having dark brown conidia (avg. length: width ≤ 1.5). *Pilidiella diplodiella*, previously *C. diplodiella*, causal organism of white rot of grapevines, was shown to be an older name for *C. petrakii*. This fungus is present in South Africa and is therefore no longer of quarantine importance. Based on analyses of the histone (H3) gene sequences of isolates in the *P. diplodiella* species complex, *P. diplodiella* was separated from a newly described species, *P. diplodiopsis*. A new species, *P. eucalyptorum*, is proposed for isolates formerly treated as *C. fragariae*, associated with leaf spots of *Eucalyptus* spp. This species clustered basal to *Pilidiella*, and may represent yet a third genus within this complex. *Pilidiella destruens* was newly described as anamorph of *Schizoparme destruens*, which is associated with twig dieback of *Eucalyptus* spp. in Hawaii.

The genus *Botryosphaeria* Ces. & De Not. are known to be cosmopolitan, with broad host ranges and geographical distributions. Several saprotrophic species have been reported from grapevines, while others are severe pathogens of this host. These species include *B. dothidea* (Moug.: Fr.) Ces. & De Not., *B. parva* Pennycook & Samuels, *B. obtusa* (Schwein.) Shoemaker, *B. stevensii* Shoemaker, *B. lutea* A.J.L. Phillips and *B. ribis* Grossenb. & Duggar. Species reported from South Africa as grapevine pathogens are *B. obtusa*, *B. dothidea*, *B. ribis* and *B.*

vitis (Schulzer) Sacc. In the present study, morphological, DNA sequence data (ITS 1, 5.8S, ITS 2 and EF1- α) and pathological data were used to distinguish 11 *Botryosphaeria* spp. associated with grapevines from South Africa and other parts of the world. *Botryosphaeria australis*, *B. lutea*, *B. obtusa*, *B. parva*, *B. rhodina* and a *Diplodia* sp. were confirmed from grapevines in South Africa, while *Diplodia porosum*, *Fusicoccum viticlavatum* and *F. vitifusiforme* were described as new species. Although isolates of *B. dothidea* and *B. stevensii* were confirmed from grapevines in Portugal, neither of these species, nor *B. ribis*, were isolated in this study. All grapevine isolates from Portugal, formerly presumed to be *B. ribis*, are identified as *B. parva* based on EF1- α sequence data. Artificial inoculations on grapevine shoots showed that *B. australis*, *B. parva*, *B. ribis* and *B. stevensii* are more virulent than the other species studied. The *Diplodia* sp. collected from grapevine canes was identified as morphologically similar, but phylogenetically distinct from *D. sarmentorum*, while *D. sarmentorum* was confirmed as anamorph of *Otthia spiraeae*, the type species of the genus *Otthia* (Botryosphaeriaceae). A culture identified as *O. spiraeae* clustered within *Botryosphaeria*, and is thus regarded as a probable synonym. These findings confirm earlier suggestions that the generic concept of *Botryosphaeria* should be expanded to include genera with septate ascospores and *Diplodia* anamorphs.

The genus *Phomopsis* (Sacc.) Bubák contains many species that are plant pathogenic or saprotrophic. Ten species are known from grapevines. However, only two have been confirmed as being pathogenic, namely *P. viticola* (Sacc.) Sacc., causal organism of Phomopsis cane and leaf spot and *P. vitimegaspora* Kuo & Leu (teleomorph *Diaporthe kyushuensis* Kajitani & Kanem.), causal organism of swelling arm disease of grapevines. *P. amygdali* (Delacr.) J.J. Tuset & M.T. Portilla, a known pathogen from *Prunus* sp., was shown to be a possible pathogen of grapevines in a previous study. *D. perijuncta* Niessl. causes bleaching of dormant canes only and is therefore of little importance as a grapevine pathogen. Recently a number of *Phomopsis* isolates were obtained from grapevines in the Western Cape province of South Africa. Isolations were made from *Phomopsis*-like symptoms, pruning wounds and asymptomatic nursery plants. These isolates showed great variation in morphology and cultural characteristics. Earlier taxonomic treatments of *Phomopsis*, based species identification on host specificity, cultural characteristics and morphology. Recent studies have indicated that these characteristics can no longer be used to distinguish species of *Phomopsis* due to wide host ranges and morphological plasticity of some species. The use of anamorph/teleomorph relationships in species identification is also untenable, since *Diaporthe* teleomorphs have only been described for approximately 20% of the known *Phomopsis* species. In this study morphological data, DNA

sequences (ITS-1, 5.8S, ITS-2) and pathogenicity data were combined to distinguish *Phomopsis* spp. from grapevines. Fifteen species of *Phomopsis* were delineated by phylogenetic analysis of ITS sequence data. *Diaporthe helianthi*, a sunflower pathogen, was reported from grapevines for the first time, with a further six, unknown species also distinguished. Three different clades contained isolates previously identified as *D. perijuncta*. Based on type studies, it appeared that the name *D. viticola* was available for collections from Portugal and Germany, a new species, *D. australafricana*, was proposed for South African and Australian isolates, formerly treated as *D. perijuncta* or *D. viticola*. An epitype specimen and culture were designated for *D. perijuncta*. This species was distinguished from *D. viticola* and *D. australafricana* based on morphology and DNA phylogeny. Artificial inoculations of green grapevine shoots indicated that, of the species tested, *P. amygdali*, a known pathogen of peaches in the USA, and *P. viticola* were the most virulent.

OPSOMMING

In 'n poging om sommige patogene geassosieer met stamsiektes en syndrome, te beveg, het die navorsing in die tesis gefokus op die taksonomie en patologiese aspekte van *Coniella/Pilidiella*, *Botryosphaeria* en *Phomopsis* spp.

Voorheen is konidium pigmentasie gebruik om *Pilidiella* (hialien tot ligbruin konidia) van *Coniella* (donkerbruin konidia) te skei. Onlangs is hierdie twee genera egter as sinoniem beskou met die ouer naam, *Coniella*, wat voorkeur gekry het. Die belangrikste spesies in die *Coniella/Pilidiella* kompleks van wingerd is *C. diplodiella* (Speg.) Petr. & Syd., die veroorsakende organisme van witvrot van wingerd. Vorige studies het dit moeilik gevind om te onderskei tussen *C. diplodiella* en *C. fragariae* (Oudem.) B. Sutton, wat bekend is dat dit in grond voorkom en ook blaarsiektes van *Fragaria* en *Eucalyptus* veroorsaak. Beide hierdie spesies is tevore in Suid-Afrika aangemeld. Geen van die aanmeldings van *C. diplodiella* is egter wetenskaplik bewys nie en daarom is dit steeds 'n kwarantyn organisme. Hierdie kwarantyn status is egter bevraagteken. Op grond van DNS volgordes van die interne getranskribeerde spasiëerder area ("ITS1", "ITS2"), die 5.8S rRNS geen, die groot ribosomale subeenheid ("LSU") en die verlengingsfaktor 1- α geen ("EF-1 α ") van die tipe spesies van *Pilidiella* en *Coniella*, is *Coniella* van *Pilidiella* geskei, met die meerderheid van die taxa wat binne *Pilidiella* resorteer. *Pilidiella* word gekarakteriseer deur spesies met hialien tot ligbruin konidia (gem. lengte: breedte > 1.5), in teenstelling met die donkerbruin konidia van *Coniella* (gem. lengte: breedte \leq 1.5). Daar is verder bewys dat *Pilidiella diplodiella*, voorheen *C. diplodiella*, veroorsakende organisme van witvrot van wingerd, die ouer naam van *C. petrakii* is. Hierdie swam is teenwoordig in Suid-Afrika en *P. diplodiella* is dus nie meer van kwarantyn belang nie. Op grond van analyses van die histoon (H3) volgordes van spesies in die *P. diplodiella* spesies kompleks, is *P. diplodiella* geskei van 'n nuut beskryfde spesie, *P. diplodiopsis*. 'n Nuwe spesie, *P. eucalyptorum*, is ook voorgestel vir isolate voorheen beskou as *C. fragariae*, geassosieer met blaarvlek van *Eucalyptus* spp. Hierdie spesie het basaal van *Pilidiella* gegroepeer en mag moontlik nog 'n derde genus binne hierdie kompleks verteenwoordig. *Pilidiella destruens* is nuut as anamorf van *Schizoparme destruens* beskryf, wat geassosieer word met loot terugsterwing van *Eucalyptus* spp. in Hawaii.

Die genus *Botryosphaeria* Ces. & De Not. is bekend as kosmopolitaans met 'n wye gasheerreëks en geografiese verspreiding. Verskeie saprofitiese spesies is aangemeld vanaf wingerd, terwyl ander ernstige patogene van hierdie gasheer is. Laasgenoemde spesies sluit in *B.*

dothidea (Moug.: Fr.) Ces. & De Not., *B. parva* Pennycook & Samuels, *B. obtusa* (Schwein.) Shoemaker, *B. stevensii* Shoemaker, *B. lutea* A.J.L. Phillips en *B. ribis* Grossenb. & Duggar. Spesies aangemeld in Suid-Afrika as wingerdpatogene, is *B. obtusa*, *B. dothidea*, *B. ribis* en *B. vitis* (Schulzer) Sacc. In hierdie studie is morfologiese, DNS volgorde data ("ITS1", "ITS2", 5.8S en "EF-1 α ") en plantpatologiese data gebruik om 11 *Botryosphaeria* spesies, geassosieer met wingerde in Suid-Afrika en verskeie ander wêrelddele, te onderskei. *Botryosphaeria australis*, *B. lutea*, *B. obtusa*, *B. parva*, *B. rhodina* en 'n *Diplodia* sp. is bevestig van wingerde in Suid-Afrika, terwyl *Diplodia porosum*, *Fusicoccum viticlavatum* en *F. vitifusiforme* as nuwe spesies beskryf is. Alhoewel isolate van *B. dothidea* en *B. stevensii* bevestig is van wingerde in Portugal, is geen van hierdie spesies en ook nie *B. ribis* geïsoleer nie. Alle isolate vanaf wingerd in Portugal, voorheen beskou as *B. ribis*, is as *B. parva* op grond van hul "EF-1 α " volgordes geïdentifiseer. Uit kunsmatige isolasies gemaak op wingerdlote is die gevolgtrekking gemaak dat *B. australis*, *B. parva*, *B. ribis* en *B. stevensii* meer virulent is as die ander spesies wat bestudeer is. Die *Diplodia* sp. versamel vanaf wingerdlote is geïdentifiseer as morfologies eenders, maar filogeneties verskillend van *D. sarmentorum*, terwyl *D. sarmentorum* bevestig is as die anamorf van *Othia spiraeae*, die tipe spesie van die genus *Othia* (Botryosphaeriaceae). 'n Kultuur wat as *O. spiraeae* geïdentifiseer is, het binne *Botryosphaeria* gegroepeer, en word dus as 'n moontlike sinoniem beskou. Hierdie bevindinge bevestig vroeëre voorstelle dat die generiese konsep van *Botryosphaeria* uitgebrei behoort te word om genera met gesepteerde askospore en *Diplodia* anamorwe in te sluit.

Die genus *Phomopsis* (Sacc.) Bubák bevat verskeie spesies wat as of plantpatogenies, of saprofities, beskryf is. Tien spesies is bekend op wingerd. Slegs twee is as patogenies bevestig, naamlik *P. viticola* (Sacc.) Sacc., veroorsakende organisme van loot-en-blaarvlek ("streepvlek") en *P. vitimegaspora* Kuo & Leu (teleomorf *Diaporthe kyushuensis* Kajitani & Kanem.), veroorsakende organisme van geswelde arm van wingerd. In 'n vroeëre studie is bevind dat *P. amygdali* (Delacr.) J.J. Tuset & M.T. Portilla, 'n bekende patogeen van *Prunus* sp., moontlik ook 'n patogeen van wingerd mag wees. *D. perijuncta* Niessl. veroorsaak egter net verbleiking van dormante lote en is dus van min belang as 'n wingerd patogeen. Gedurende die afgelope twee jaar is verskeie *Phomopsis* isolate van wingerde in die Wes-Kaap provinsie van Suid-Afrika verkry. Isolاسies is gemaak van *Phomopsis*-agtige simptome, snoeiwonde en asimptomatiesse kwekeryplante. Die isolate verkry uit hierdie materiaal het groot variasie ten opsigte van morfologie en kultuureienskappe getoon. Vroeëre taksonomiese verhandelings van *Phomopsis* het spesies-identifikasie op gasheerspesifisiteit, kultuureienskappe en morfologie gebaseer. Onlangse studies het egter getoon dat, weens wye gasheerreëse en morfologiese plastisiteit van

sommige spesies, hierdie eienskappe nie meer gebruik kan word om *Phomopsis* spesies te identifiseer nie. Die gebruik van anamorf/teleomorf verwantskappe in die identifikasie van *Phomopsis* spesies ook onbruikbaar omdat *Diaporthe* teleomorwe vir slegs ongeveer 20% van die bekende *Phomopsis* spesies beskryf is. Die huidige studie het dus morfologiese data, DNS volgordes ("ITS1", 5.8S, "ITS2") en patogenisiteitsdata gekombineer ten einde *Phomopsis* spp. vanaf wingerd te identifiseer. Vyftien *Phomopsis* spesies is deur die filogenetiese analise van die interne getranskribeerde spasieerder area ("ITS") volgordes geskei. *Diaporthe helianthi*, 'n bekende patogeen van sonneblomme, is vir die eerste maal op wingerd aangeteken, terwyl 'n verdere ses, tans onbekende spesies van *Phomopsis* ook geïdentifiseer is. Drie verskillende groepe het isolate bevat wat voorheen as *D. perijuncta* geïdentifiseer is. Gebasseer op studies van tipes, het dit voorgekom dat die naam *D. viticola* beskikbaar is vir isolate uit Portugal en Duitsland. 'n Nuwe spesie, *D. australafricana*, is voorgestel vir Suid-Afrikaanse en Australiese isolate wat voorheen behandel is as *D. perijuncta* of *D. viticola*. 'n Epitepe monster en kultuur is vir *D. perijuncta* benoem. Hierdie spesie is van *D. viticola* en *D. australafricana* onderskei op grond van morfologie en DNS filogenie. Kunsmatige inokulasies van groen wingerdlote het getoon dat *P. amygdali*, bekende perske patogeen, en *P. viticola* die mees virulent was.

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1. REVIEW OF GRAPEVINE DISEASES CAUSED BY SPECIES OF *CONIELLA*, *BOTRYOSPHERA* AND *PHOMOPSIS*

INTRODUCTION

In South Africa, grapevine cultivation forms an integral part in the contribution that agriculture makes towards the economy of the country. Wine grape vineyards cover an area of 106 331 ha and there are approximately 4390 producers and 388 wine cellars, directly employing more than 350 000 people. The total number of people that the wine industry thus sustains could be as much as four times the abovementioned number (<http://www.sawis.co.za>). The value of the industry is, however, not confined to job creation. Annually approximately 746 million litres of wine are produced of which 180 million litres are exported. Total producer's income is R 1.6 billion (<http://www.sawis.co.za>). It is thus clear that this industry is of great importance to South African agriculture, as well as the economy of the country.

Like most agricultural crops, grapevines are host to a range of different pathogens. The diseases caused by these pathogens lead to variable amounts of losses each year. The research in this thesis focuses on some important trunk diseases of grapevines that are caused by species of *Coniella*, *Botryosphaeria*, and *Phomopsis*. The diseases associated with these pathogens, the symptoms they cause, as well as possible control measures are briefly discussed below.

WHITE ROT OF GRAPEVINES

Introduction

White rot of grapevines was first described in Italy in 1878 (Bisiach, 1988). It is commonly accepted that the disease originated from the United States and spread to other grape-producing areas of the world and is currently widely distributed in close relationship with the occurrence of *Vitis vinifera* L. (Matthee & Thomas, 1981; Crous & Carstens, 2001). This disease is commonly known to occur when grapevines are damaged after hailstorms. The fungus, which commonly infects through wounds, can cause losses of between 20–80% (Bisiach, 1988).

Symptoms

Infection is characterised by berries that turn yellowish, later becoming pale pink-blue in colour, losing their turgidity. As the infection progresses, berries become covered with pycnidia. The pycnidia raise the cuticle of the berry, which allows air to penetrate beneath the cuticle giving the berry a whitish colour. The pycnidia turn grey-white as they mature, hence the name white rot (Bisiach, 1988).

Under favourable conditions (high temperature and relative humidity) the disease can spread systemically from the infected berries to the pedicel and rachis of the cluster, which might lead to the destruction of a large part of the cluster (Bisiach, 1988). When uninjured berries are infected, they turn pink-blue in colour, with the discolouration starting at the pedicel. Depending on weather conditions, pycnidia may develop on the surface of the berry (very humid conditions) or inside the berry on the seeds (dry conditions). At the end of the season these infected berries fall to the ground and act as a source of inoculum for infections in the following season (Bisiach, 1988).

Lesions that develop on the main rachis cause the distal portion of the cluster below to dry. Berries on young, immature clusters turn pale green and flaccid when infected, and brown as the disease develops. It has been reported that white rot may cause canker formation on non-lignified shoots, but this was mostly recorded on nursery plants (Bisiach, 1988).

Etiology

The causal organism of white rot of grapevines is *Coniella diplodiella* (Speg.) Petr. & Syd. This organism has a wide geographical distribution and host range (Bisiach, 1988). Other species of *Coniella*, with wide host ranges, were also reported on grapevines. Some of these species are known to occur in South Africa. However, these species were reported on other hosts and not on grapevines. Species that are of relevance to grapevines are *C. petrakii* B. Sutton, *C. fragariae* (Oudem.) B. Sutton (Crous *et al.*, 2000) and *C. granati* (Sacc.) Petr & Syd. When pathogenicity studies were done using these species of *Coniella*, it was found that isolates of *C. petrakii* and, to a lesser extent *C. fragariae*, could also cause white rot symptoms (Tiedemann, 1985).

Epidemiology

Coniella diplodiella typically infects the berry clusters before véraison when hailstorms might cause damage and wounds for infection. Infection might, however, also be initiated by rain-

splashed soil. The pathogen requires temperatures of 15°C or more to develop and conidia germinate optimally between 24°C and 27°C (Bisiach, 1988; Crous & Carstens, 2001). The disease can therefore occur in vineyards subjected to summer rain, followed by high relative humidity for prolonged periods of time.

Management

Control of white rot is based on a number of strategies. The most important one is the prevention of wounds and the use of trellis systems that keep the grapevine shoots and grape bunches well above ground level. Many fungicides, with active ingredients such as captab, dichlofluanid and chlorothanil, are effective in the control of white rot. Folpet or captab, provide about 75% control if applied within 12–18 h after a hailstorm. If this application of fungicide is, however, delayed for 21 h, the efficacy declines to 50%, and after 24 h it is totally ineffective, especially if the temperature is above 20°C. Dicarboximides also provide good control, provided that the temperature is below 20°C and it is applied within 12–18 h after a hailstorm (Bisiach, 1988). Folpet, a non-systemic contact fungicide, can also be applied as a preventative spray (Gualco & Togni, 2001).

Sanitation in the vineyards can also help in lowering the inoculum levels and can be done by removing and burning all pruning debris that fall on the ground and might harbour inoculum that could cause subsequent infections (Matthee & Thomas, 1981; Bisiach, 1988).

South African perspective

In South Africa, white rot, caused by *C. diplodiella*, has twice been reported. The first report was made in 1977 by Verbeek in the Annual Report of the Secretary for Agricultural Services. Matthee and Thomas (1981) published the second report of this disease. However, in neither of these reports was the morphology of the fungus described, nor any reference material lodged in the National Collection of Fungi in Pretoria (PREM). Thus, it was never scientifically proven that this fungus occurred in South Africa. It was consequently given quarantine status and no grapevine material infected with this fungus may enter the country (Crous & Carstens, 2001). In the recent past, however, the listing of *C. diplodiella* as a quarantine organism has led to much controversy as it caused the rejection of a number of consignments of grapevine propagation material that were imported from France and Australia. It was argued that the reports by Verbeek (1977) and Matthee and Thomas (1981) were sufficient proof for the removal of the fungus from the South African phytosanitary lists.

Recently, *Coniella* was also isolated from roots of grapevine nursery plants in South Africa. These isolates were identified as *C. petrakii*, a fungus that has previously been reported from South Africa on *Eucalyptus* (Crous *et al.*, 2000). To further add to the confusion surrounding this pathogen, Sutton (1980) treated *C. diplodiella* as a synonym of *C. fragariae*. A systematic reappraisal of the genus *Coniella* is therefore urgently required to resolve this matter.

BOTRYOSPHAERIA DISEASES OF GRAPEVINES

Introduction

In recent years it has become a well-known fact that the genus *Botryosphaeria* Ces. & De Not. has a very wide host range and that species can occur as saprobes, parasites or endophytes. Some species are known to cause cankers, dieback and various other diseases. Many of these species have also been reported from grapevines and have been associated with a wide variety of symptoms (Phillips, 2002; Table 1). These symptoms are especially severe in cases where the host plant is subjected to stress (Boyer, 1995). Confusing overlap of symptoms between diseases is caused by the fact that different disease names are given for certain sets of symptoms in different countries (Hewitt, 1988; Philips, 1998; Larignon & Dubos, 2001; Leavitt, 2003). Species occurring on grapevines in different countries also differ in pathogenicity and this has led to further confusion as to which species of *Botryosphaeria* are the important pathogens of grapevines (Phillips, 2002).

Diagnosis of *Botryosphaeria* diseases is furthermore problematic, since symptoms occurring on grapevines in the field closely resemble that of other diseases such as dead arm caused by *Phomopsis viticola* (Sacc.) Sacc. and Eutypa dieback caused by *Eutypa lata* (Pers.) Tul. & C. Tul. (= *Eutypa armeniacae* Hansf. & M.V. Carter (Castillo-Pando *et al.*, 2001). Accurate identification of the causal species is also difficult since the teleomorphs of *Botryosphaeria* species are very seldom encountered in nature, and rarely form in artificial cultures. The diversity among these teleomorphs is furthermore insufficient to allow clear differentiation at species level. This has brought about that taxonomy and identification of *Botryosphaeria* species are based mainly on characters of the anamorph (Phillips, 2002).

The anamorph genera of *Botryosphaeria* have also been the subject of significant revision. The primary reason for this revision is that these genera were not clearly defined when they were initially described, and that up to seven genera have subsequently been introduced. After the work

of Crous and Palm (1999), Denman *et al.* (2000) and Phillips (2000), many of the problems surrounding these genera have been resolved.

Symptoms

Although the importance of the various *Botryosphaeria* species occurring on grapevines has not yet been clarified, various symptoms associated with different *Botryosphaeria* species have been described on grapevines worldwide (Table 1). These symptoms develop slowly and lead to a gradual decline in vigour and yield (Phillips, 1998). This aspect is probably the reason why the most severe losses due to this disease occur in grapevines that are eight years and older (Larignon & Dubos, 2001).

A symptom that is often associated with *Botryosphaeria* species is bud mortality, which leads to a reduction in yield, breakage of branches and shoot dieback. Bud necrosis and shoot dieback are often the result of young shoots being infected by the fungus early in the season. The infection develops into elongated black lesions on the internodes. The infected branches become swollen at the base, with the blackened cortex eventually rupturing. These shoots are consequently very brittle and can easily collapse under their own weight. Some of the branches, which do not break, may die back. Later in the season, after harvest, the black lesions turn grey or white with black fruiting structures immersed in the host tissue (Phillips, 1998). These shoots have a bleached appearance, similar to Phomopsis cane blight (Phillips, 2002).

Pruning wound infections lead to trunk and arm dieback, with a dark brown discolouration of the wood that starts at the pruning wounds and spreads down the trunk. In some cases brown streaking of the wood is also encountered with a dark brown discolouration of the trunk. In cross-sectioned trunks and arms, the streaking has the appearance of small black spots. This symptom is similar to the symptom found in trunks and arms affected by Petri disease (= Black Goo), which is caused by *Phaeomoniella chlamydospora* (W.Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams. It differs from Petri disease, however, in that the spots are more diffuse and, in the case of Petri disease, a black tarry substance oozes from the spots, which is not the case with the spots caused by *Botryosphaeria* species (Castillo-Pando *et al.*, 2001; Phillips, 2002). Another internal wood symptom that can be seen in cross-sectioned arms and trunks is a V-shaped necrotic sector. This symptom is also found in association with brown wood streaking. This symptom resembles Eutypa dieback, but can be distinguished by the absence of poor shoot development and small, yellow, malformed leaves (Castillo-Pando *et al.*, 2001).

Infection by *Botryosphaeria* species can also manifest itself by causing mild chlorosis on the leaves, depending on how far the fungus has invaded the vascular tissue. Infection of berries does not occur often, but severe berry and cluster rot have been reported from White and Red Hanepoot cultivars in South Africa (Lehoczy, 1974, 1988). Fruit rot symptoms associated with *Botryosphaeria* species were also reported from France (Larignon & Dubos, 2001) and the USA (Hewitt, 1988; Milholland, 1988, 1991; Kummuang *et al.*, 1996a, 1996b; Leavitt, 2003). When berries become infected, they initially appear water-soaked, and as the rot develops and the skin cracks, the berries become covered in a white mass of mycelium. The berries eventually dry out and become mummified with black pycnidia emerging on the surface. The dried-out berries are seldom seen, due to the fact that they become infected with secondary fungi and yeasts that cause the cluster to turn into a rotten mass known as summer bunch rot (Hewitt, 1988).

In cases where isolations were made from young plants where the graft union failed, *P. chlamydospora* was mostly isolated, but in some cases various *Botryosphaeria* species were also isolated from the failed graft unions (Phillips, 2002).

Etiology

As stated earlier, the *Botryosphaeria* species reported as grapevine pathogens are associated with more than one type of symptom and disease. The different *Botryosphaeria* species and the diseases they are associated with are briefly described below.

***Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not.** One of the diseases that *B. dothidea* is associated with is excoriose. This disease is especially well known in Portugal and France (Phillips, 1998; Larignon & Dubos, 2001). For many years *Phomopsis viticola* was regarded as the causal organism of excoriose in the USA. In Europe, the causal organism was regarded as *Macrophoma flaccida* (Viala & Ravaz) Cavara. After *P. viticola* was reported from Germany, this fungus was considered as the sole cause of excoriose and dead arm in Europe and the USA. In 1997, Phillips and Lucas (1997) showed that *M. flaccida* is synonymous with *Fusicoccum aesculi* Corda, which is the anamorph of *Botryosphaeria dothidea*.

Macrophoma flaccida was also found to be widely distributed in association with excoriose in Portugal (Tomaz & Rego, 1990). This led to the conclusion that the cause of excoriose may be *B. dothidea*, previously known as *M. flaccida*. However, doubt still remained as to the pathogenicity of *B. dothidea* on grapevines. This led to the study of Phillips (1998; 2000) where the pathogenicity of *B. dothidea* and *P. viticola* were tested in Portugal. The results showed that the symptoms caused by the two fungi are very similar and that these symptoms corresponded with

the symptoms commonly associated with excoriose. This confirmed that *B. dothidea* (previously *M. flaccida*) was indeed a causal organism of excoriose (Phillips, 1998; 2000).

In Portugal, *B. dothidea* was also reported as one of the *Botryosphaeria* species isolated from failed graft unions of young plants (Phillips, 2002). In the USA, *F. aesculi*, the anamorph of *B. dothidea*, was reported as the causal organism of Macrophoma rot of Muscadine grapes (Milholland, 1988, 1991; Kummung *et al.*, 1996a, 1996b).

***Botryosphaeria parva* Pennycook & Samuels.** In Portugal, *B. parva* is associated with grapevine decline syndrome. It was isolated from more than one type of symptom. The first symptom was trunk dieback, with dark brown discoloration of the wood. It was also isolated from the symptom that resembles the small black spots that is characteristic of Petri disease and can be seen when looking at a cross section of the trunk and arms of the grapevine. In some cases it was isolated from dead tissue surrounding necrotic buds (Phillips, 2000; Castillo-Pando *et al.*, 2001; Phillips, 2002).

***Botryosphaeria obtusa* (Schwein.) Shoemaker.** *Botryosphaeria obtusa* occurs on a wide variety of woody hosts and can live saprophytically on dead wood and bark (Castillo-Pando *et al.*, 2001). It has been recognised as a wound pathogen and can cause dieback symptoms as well as cankers. In Portugal, *B. obtusa* was found associated with reduced growth, reduced bud burst and death of arms (Phillips, 2002).

This species is also associated with grapevine decline syndrome and was isolated from more than one type of symptom. Similar to *B. parva*, *B. obtusa* was also isolated from the Petri disease-like spots (Castillo-Pando *et al.*, 2001; Phillips, 2002). It also caused decline or dieback in young plants where it was isolated from failed graft unions, similarly to *B. dothidea* and *B. parva* (Phillips, 2002).

Another symptom type found in association with *B. obtusa* is a V-shaped necrotic sector that can be seen in the trunk and arms of the grapevine in cross section. This symptom is also found in association with brown wood streaking. In Australia, *B. obtusa* was also found to be the causal organism of dieback of Semillon grapevines in New South Wales (Castillo-Pando *et al.*, 2001).

***Botryosphaeria stevensii* Shoemaker.** *Botryosphaeria stevensii* is another of several species of *Botryosphaeria* that are associated with grapevine decline syndrome. Similar to *B. parva* and *B. obtusa*, it was in some cases isolated from the small black spots that resemble the spots commonly associated with Petri disease (Castillo-Pando *et al.*, 2001; Phillips, 2002).

Isolations made from dead tissue surrounding dead buds often yielded *B. stevensii*, with *B. parva* also encountered in some cases (Phillips, 2002).

B. stevensii is also associated with another disease, black dead arm, which was first described in Hungary in 1974, and is a well known disease of grapevines in France. In the case of this disease, *B. stevensii* causes leaf chlorosis, wilting and in some cases fruit rot (Lehoczky, 1974, 1988; Larignon & Dubos, 2001). In Australia, *B. stevensii* was also isolated from grapevines showing decline symptoms (Pascoe, 1998).

***Botryosphaeria lutea* A.J.L. Phillips.** This species, like others discussed by Phillips (1998; 2000; 2002), is also associated with grapevine decline syndrome. It was also isolated, like *B. obtusa* and *B. stevensii*, from the Petri disease-like spots that are visible in the cross section of the trunk or arms (Castillo-Pando *et al.*, 2001; Phillips, 2002). Similar to *B. parva* and *B. obtusa*, it was also isolated from bleached, dormant canes that resemble the bleached canes normally associated with Phomopsis cane and leaf spot (Phillips, 2002).

***Botryosphaeria ribis* Grossenb. & Duggar.** In Australia, this species was mostly associated with fruit rot symptoms in vineyards and it was concluded that it might not be a pathogen of shoots or canes in that country (Pascoe, 1998). However, it might also be involved with some of the other symptoms listed in Table 1.

***Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx.** *Botryosphaeria rhodina* is also associated with grapevine decline syndrome. In Australia and California, USA, it was isolated from the necrotic V-shaped symptom that is associated with grapevine decline syndrome in Portugal (Pascoe, 1998; Phillips, 2002.)

In the USA, the anamorph of *B. rhodina*, *Lasiodiplodia theobromae* (Pat.) Griffiths & Maubl., which was previously known as *Diplodia natalensis* Pole-Evans, is regarded as the causal organism of cane dieback and bunch rot symptoms known as Diplodia cane dieback and bunch rot. *L. theobromae* is also associated with other symptoms of this disease, which includes lesion development on the shoots that could spread to the arms, causing dead arm symptoms (Hewitt, 1988).

Epidemiology

The symptoms attributed to the various *Botryosphaeria* species are very similar (Table 1). However, the epidemiology might be slightly different for each disease. The epidemiology of the various diseases is briefly discussed below.

Excoriose. This disease is present wherever grapevines are grown. Infection of the young grapevine shoots occurs early in the season and is severe when wet weather is encountered (Phillips, 1998).

Black Dead Arm. *Botryosphaeria stevensii*, the causal organism, overwinters in the diseased wood of grapevines, with pycnidia developing in spring and autumn during rainy weather. Infection occurs through pruning wounds or wounds caused by mechanical injury. The optimal temperature for infection is between 23–26°C (Lehoczky, 1988). Infection of grapes occurs near ripening (Lehoczky, 1974, 1988).

Macrophoma rot. *Botryosphaeria dothidea*, the causal organism, overwinters as pycnidia on dried-out berries on the vineyard floor. This disease develops optimally at 28°C (Milholland, 1988, 1991; Kummuang *et al.*, 1996a, 1996b).

Diplodia Cane Dieback and Bunch Rot. *Diplodia* cane dieback and bunch rot is generally a disease that is most severe in areas with warm to hot weather. It is also favoured by high relative humidity and occasional summer rain (Hewitt, 1988). In vineyards, the fungus overwinters inside diseased parts of the grapevine or in the soil. In spring, when mean temperatures start to rise and the grapevine becomes active, the propagules of *L. theobromae* also become active. The fungus can become soilborne from pruning debris lying on the vineyard floor. The disease is spread by windblown conidia or splash-dispersed in water droplets from rain or sprinkle irrigation. Infection of shoots occurs when the shoots touch wet soil or are covered with soil during cultivation. The fungus then spreads along the shoot and may grow into arms and spurs of the plant and kill tissue as it grows. Berry infection occurs during or shortly after bloom from windblown conidia (Hewitt, 1988).

Management

The management of *Botryosphaeria* diseases is very difficult, as the information on especially chemical control is very limited. In many instances, recommended control measures vary from one country to another and even from one region to another (Milholland, 1991).

France is one of the very few countries where a fungicide is registered for control of black dead arm, which is caused by *B. obtusa*, *B. dothidea* and in some cases *B. stevensii*. Sodium arsenite is recommended and must be used under the same conditions as for the treatment of Esca disease (Lehoczky, 1974, 1988; Larignon & Dubos, 2001). However, the use of sodium arsenite as a fungicide in agriculture, especially viticulture, has been greatly reduced since it has been banned in France as is the case in many other countries (<http://www.inra.fr/presse>). The

environmental pollution caused by sodium arsenite, and the build-up of arsenite in crops sprayed with sodium arsenite, is also a major concern and was one of the main reasons for it being banned. The biggest concern was that the high levels of arsenite in crops might be a danger to consumer health (Decoin, 2001; Lyubun *et al.*, 2002). Another country where a *Botryosphaeria* disease is controlled by a fungicide, is the USA where Macrophoma rot (*B. dothidea*) is controlled by the application of protective fungicides like maneb, which start after bloom and continues throughout the fruit-ripening period (Milholland, 1988).

Another control measure that is widely recommended for most of the diseases caused by the various *Botryosphaeria* species is good sanitation practices. After pruning, debris should be removed from the vineyard and burnt. When the disease has not spread throughout the whole plant the diseased parts can be cut off and removed. Whole infected plants can also be removed from the vineyard.

To prevent the occurrence of Diplodia cane dieback and bunch rot it is important to prune and train grapevines in such a manner that the shoots do not touch the ground. It is also important to prevent unnecessary wounding of plants as most of the *Botryosphaeria* species which occur on grapevines, are regarded as wound pathogens (Lehoczky, 1988; Milholland, 1988, 1991; Larignon & Dubos, 2001).

As it is well known that *Botryosphaeria* is mainly a wound pathogen, much of the research into the management of *Botryosphaeria* diseases of grapevines has been aimed at the protection of pruning wounds with fungicides and/or biological control agents (Larignon & Dubos, 2001). In trials done by Leavitt (2003), it was shown that iprodione, benomyl, captan and penconazole were all effective in reducing infection of pruning wounds by *L. theobromae* if it were applied prior to inoculation in the glasshouse. It is, however, important to use these fungicides alternately as it has been shown that resistance can develop towards iprodione (ZhongHau *et al.*, 2000).

South African perspective

In South Africa, three species of *Botryosphaeria* have previously been reported from grapevines. These are *B. ribis*, *B. dothidea* and *B. obtusa* (Crous *et al.*, 2000). In the recent past, the prominence of *Botryosphaeria* as an important pathogen of grapevines in South Africa has come to the fore. This was clearly shown by the fact that *Botryosphaeria* species were isolated from 9% of all diseased grapevines received for diagnostic analysis by the Disease Management Division of the Agricultural Research Council's institute, Infruitec-Nietvoorbij in Stellenbosch (Fourie & Halleen, 2001). The fungus was isolated from a variety of symptoms similar to that

described by Phillips (2001) for grapevine decline syndrome. Isolations were also made from asymptomatic nursery grapevine plants. The isolates could, however, not be identified due to the fact that *Botryosphaeria* does not readily form fruiting structures in culture. It thus became necessary to identify and characterise the South African isolates in order to determine which species are associated with grapevines and to test their pathogenicity in order to determine which are the important pathogens of grapevines. If this is known, further research could then focus on the epidemiological aspects of the important pathogens only. This would ultimately lead to the development of management strategies aimed specifically at these species.

PHOMOPSIS DISEASES OF GRAPEVINES

Introduction

Two taxa from the genus *Phomopsis* are most frequently associated with grapevines. These are *Phomopsis viticola* (Sacc.) Sacc., also known as *Phomopsis* Taxon 2, and *Phomopsis* Taxon 1 (Mostert *et al.*, 2001; Rawnsley & Wicks, 2002a). Up to very recently the exact role of *Phomopsis* Taxon 1 in disease development was unclear (Rawnsley *et al.*, 2001). Although Taxon 1 was often isolated from dead buds no association could be found between the presence of Taxon 1 infection and bud mortality, as Taxon 1 was also isolated from healthy buds (Rawnsley *et al.*, 2001; Rawnsley & Wicks, 2002a). It has, however, been confirmed that this fungus only causes bleaching of canes in winter. The bleaching appears white with small black spots visible on the surface of the cane and is caused by pycnidia pushing through the cuticle of the cane, thereby admitting air underneath and giving the cane a bleached appearance (Rawnsley *et al.*, 2001). *Phomopsis* Taxon 1, however, appears to be of minor pathogenic importance on grapevine. On the other hand, *P. viticola* (Taxon 2) is an important and well-known pathogen of grapevine and causes *Phomopsis* cane and leaf spot. A new disease, swelling arm of grapevines, has recently been described from grapevines in Japan and Taiwan. This disease is caused by a relatively unknown *Phomopsis* species, *P. vitimegaspora* Kuo & Leu, and is characterised by swollen nodes on the arms of the grapevine as well as black lesions which girdle new, green shoots (Kuo & Leu, 1998; Kajitani & Kanematsu, 2000). These two diseases are briefly discussed below.

Phomopsis cane and leaf spot of grapevines

This is a very important disease in most parts of the world where grapevines are grown. Infection by the causal pathogen can lead to serious damage to the grapevines and subsequent losses in yield can be as high as 20–30% (Swart & De Kock, 1994; Merrin *et al.*, 1995). Over the years this disease has been confused with some other diseases of grapevines. The reason for this is that the symptoms in the field closely resemble symptoms of other diseases such as Eutypa dieback, caused by *Eutypa lata* (Pers.) Tul. & C. Tul. and disease symptoms caused by *Botryosphaeria* species (Castillo-Pando *et al.*, 2001).

Symptoms. First symptoms appear as small brown to black spots, with yellow halo's on leaves. As lesions develop, infected leaves become malformed and the necrotic spots may drop out of the leaf, giving the leaves a shot-hole appearance (Hewitt & Pearson, 1988; Swart & De Kock, 1994; Merrin *et al.*, 1995).

The first symptoms on shoots appear as small, dark lesions that enlarge and later form big dark lens-shaped blotches. These lesions occur mostly on the first three internodes of shoots. If infection is very severe, shoots or portions of shoots may die back. Shoot infection may also spread to the bunch stalks, causing it to become brittle and break, leading to a loss in yield. Bunch infections may also cause berries to turn black and dry out. The symptoms become less evident as the season progresses due to the development of new shoots and leaves that obscure these symptoms (Hewitt and Pearson, 1988; Swart & De Kock, 1994; Merrin *et al.*, 1995; Rawnsley & Wicks, 2002a).

Etiology. *Phomopsis viticola* (Taxon 2) has long been recognised as the causal organism of Phomopsis cane and leaf spot (Hewitt & Pearson, 1988; Phillips, 1999; Mostert *et al.*, 2001; Rawnsley & Wicks, 2002a).

Epidemiology. The disease is especially severe in areas where rainfall occurs after bud break, keeping the grapevines wet for prolonged periods of time (Hewitt & Pearson, 1988). Infection can lead to serious damage to the grapevines and subsequent losses in yield can be as high as 20–30% (Swart & De Kock, 1994; Merrin *et al.*, 1995).

Phomopsis viticola overwinter in the bark of infected shoots, in the form of mycelium and pycnidia. The pycnidia usually reach maturity during late winter to early spring. At this time, if wet weather occurs, conidia are released from the pycnidia and are dispersed by rain or irrigation water. Two types of conidia, alpha and beta, are formed and may be released by the pycnidia. The α -conidia cause primary infections (Hewitt & Pearson, 1988; Swart & De Kock, 1994; Merrin *et al.*, 1995). Until recently the function of β -conidia was unclear and they were believed to be sterile

and unable to cause symptoms. Sergeeva *et al.* (2003) have, however, shown that β -conidia can germinate and infect grapevine leaves, causing necrotic spots that are similar to the spots caused by α -conidia infection.

Alpha conidia germinate across a wide range of temperatures (1–37°C) and at the optimum temperature of 23°C. Infection may take place within a few hours if free water is present or if the relative humidity is near or at 100%. Usually the first tissue infected is new, green vegetative growth. The first symptoms appear 21–30 days after infection. When the weather gets very hot, the fungus becomes inactive and resumes activity in late summer to early autumn, when it becomes cooler again and some rain may fall. In the cooler areas the fungus may also stay active throughout the year (Hewitt & Pearson, 1988; Swart & De Kock, 1994).

The disease can reach epidemic proportions if rain occurs for a number of consecutive days, with temperatures in the optimal range for disease development. These conditions usually occur in early spring, when shoot growth is slow and young shoots (3–10 cm long) are especially susceptible to infection. It was also shown that fruit infection can occur very early in the season and that this infection remains latent until the fruit start to ripen. The grape berries and bunch rachis remain susceptible to infection throughout the growing season (Erincik *et al.*, 2001). If the infected wood is not removed from the vineyard, the disease severity will increase with each cool, wet spring due to the high inoculum pressure within the vineyard. Disease spread from one grapevine to another is very localised, with the disease only spreading to grapevines very close to the initial inoculum source (Hewitt & Pearson, 1988; Swart & De Kock, 1994).

Management. Management of Phomopsis cane and leaf spot rests primarily upon two strategies that can be used in combination or individually (Hewitt & Pearson, 1988). These are sanitation practices and a preventative chemical control strategy.

Sanitation practices comprise the removal of any diseased plant material after pruning. Pruning debris should preferably be removed from the vineyard and burnt. If it cannot be burned it can also be cut up and ploughed into the soil (Hewitt & Pearson, 1988). In some cases, sanitation practices are difficult to justify because it can be very labour intensive and the results of good sanitation practices are not immediately apparent (Gadoury *et al.*, 1994). The aim of sanitation is, however, to reduce the source of inoculum in the vineyard that can cause new infections and the value of good sanitation will only become apparent in following seasons.

It has also been shown that hedged grapevines that are mechanically pruned have a higher incidence of Phomopsis cane and leaf spot than grapevines that are hand-pruned. This can be contributed to a higher inoculum potential in these vineyards, since the grapevines have more

nodes and dead canes upon which pycnidia could develop and thus act as a source of inoculum for new infections. It is thus recommended that extra care in removing dead canes should be taken in mechanically pruned vineyards, thereby reducing the level of inoculum that can cause new infections on young shoots (Pscheidt & Pearson, 1989a).

A preventative chemical control strategy is recommended in areas where the disease is prevalent. The best results were obtained when more than one spray were applied. The first application should be a dormant spray with fungicides such as dithianon, benomyl, fluazinam and mancozeb. This reduces the level of inoculum at the beginning of the new growing season (Castillo-Pando *et al.*, 2001; Nair *et al.*, 2000; Rawnsley & Wicks, 2002b). This dormant spray, applied before budburst, will protect the young, green buds (Willison *et al.*, 1965; Hewitt & Pearson, 1988; Swart & De Kock, 1994). Fungicides such as captab, folpet, maneb, dithianon or mancozeb can be applied at 1–3 cm shoot length and again at 6–12 cm shoot length as new, green vegetative growth is most susceptible to infection by *P. viticola* (Willison *et al.*, 1965; Hewitt & Pearson, 1988; Swart & De Kock, 1994).

When relying on fungicide applications for control of Phomopsis cane and leaf spot there are some factors that need to be considered. If rain falls shortly after the application of a fungicide, the application must be repeated and if the weather is cool and shoot growth is slow more sprays are needed in order to protect new growth (Hewitt & Pearson, 1988). Rain shortly before the application of fungicides also reduces the effectiveness of the application, since rain causes mature pycnidia to release large numbers of conidia that can cause new infections and fungicide residue is washed off (Swart & De Kock, 1994). Pscheidt and Pearson (1989b) found that fungicide applied during bloom reduces the severity of fruit rot, as the fruit infections already occur during bloom.

It is, however, important to keep on looking for new control measures as many fungicides are taken from the market and lose their registration due to resistance developing and danger to people and animals (Gadoury *et al.*, 1994).

Swelling arm disease of grapevines

This disease of grapevines was first described in Japan in the 1960's and has since also been reported to occur on grapevines in Taiwan. At first, this disease was confused with Phomopsis cane and leaf spot of grapevines and Eutypa dieback, caused by *Eutypa lata* (Kajitani & Kanematsu, 2000). The causal organism of this disease is *Phomopsis vitimegaspora* Kuo & Leu, and its teleomorph *Diaporthe kyushuensis* Kajitani & Kanematsu.

Symptoms. The symptoms of this disease occur on all parts of the grapevine. The first symptoms are the appearance of small black spots at the base of new, green shoots. These spots become larger and coalesce to form a blackened zone at the base of the shoot, which is girdled and shoot blight consequently occurs. Oblong to elliptic shaped lesions can also develop on other parts of green shoots. After two or more years, flat, slightly hypertrophied nodes appear on canes (Kuo & Leu, 1998; Kajitani & Kanematsu, 2000). Cankers are noticed on arms of grapevines that are four or more years old. Internal wood symptoms are also visible on arms as dark brown necrotic lesions with distinct margins. These necrotic lesions could extend deeply into the wood, reaching the pith in most cases (Kuo & Leu, 1998).

Epidemiology. Infection of new, green shoots occurs between May and September (northern hemisphere), with the most important period for infection being during June and July. Infection occurs via direct penetration of the epidermis of green shoots by α -conidia. This fungus cannot infect mature wood of grapevines unless wounds are present (Kuo & Leu, 1998; Nasu *et al.*, 2003). Within a vineyard the disease is spread mainly via water-splashed conidia. Between vineyards the disease can be spread via infected nursery plants (Nasu *et al.*, 2003).

Management. Fungicide applications are recommended as control measure for the black spots and dark lesions that develop on green shoots. The same fungicides that are used for the control of *Phomopsis* cane and leaf spot can be used to control swelling arm of grapevines. These applications are, however, ineffective to control the swollen node and canker symptoms. The reason for the lack of control might be because the fungus are located internally in the wood and are thus shielded from applied fungicides. It is thus also recommended that hot water treatment should be applied to rootstock and scion material before grafting (Nasu *et al.*, 2003). Due to the fact that *Phomopsis* cane and leaf spot is such an important disease of grapevines in South Africa, several fungicides are registered in this country for the control of this disease. The fungicides registered are copper oxychloride, folpet, mancozeb and propineb, fosetyl-Al/mancozeb, for use only on table grapes, with polysulphide sulphur the only fungicide registered as a preventative dormant spray (Nel *et al.*, 2003).

South African perspective

In South Africa, *Phomopsis* cane and leaf spot has been a known disease since 1935 (Du Plessis, 1938) and was reported as a very serious pathogen of grapevines in 1958 (Synnott, 1958). Little was, however, known about the species of *Phomopsis* associated with this disease in South Africa. This situation changed after the study of Mostert *et al.* (2001) when various *Phomopsis*

species occurring on grapevines in South Africa were characterised. This study found that that *Phomopsis* species associated with diseased grapevines in the Western Cape province of South Africa were *Phomopsis* sp. 1, *Phomopsis amygdali* (a known pathogen of peaches in the United States), *Diaporthe perijuncta* (a teleomorph of a *Phomopsis* sp.), and *Phomopsis viticola* (Farr *et al.*, 1999; Scheper *et al.*, 2000; Mostert *et al.*, 2001). Pathogenicity tests done in this study furthermore revealed that *P. viticola* and *P. amygdali* caused severe lesions on inoculated grapevine shoots. *Phomopsis* sp.1 and *D. perijuncta* formed very small lesions. It was thus concluded that *P. viticola* and *P. amygdali* are important pathogens of grapevines and that *Phomopsis* sp.1 and *D. perijuncta* are less important pathogens of grapevines in South Africa (Mostert *et al.*, 2001). *Phomopsis amygdali* was, however, isolated once only from grapevines in South Africa (Mostert *et al.*, 2001).

Recently, unknown *Phomopsis* species were isolated from symptoms atypical of *Phomopsis* cane and leaf blight or swelling arm disease (Fourie & Halleen, 2001). Some of these species were isolated from severe wood decay symptoms, which led to premature decline and dieback of the affected grapevines. The possible role of *Phomopsis* species in grapevine trunk diseases should therefore be investigated.

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Table 1. Symptoms associated with different *Botryosphaeria* species

Symptoms	<i>B. dothidea</i>	<i>B. parva</i>	<i>B. obtusa</i>	<i>B. stevensii</i>	<i>B. lutea</i>	<i>B. ribis</i>	<i>B. rhodina</i>	Reference
Bud mortality	X							Phillips, 1998; Larignon & Dubos, 2001
Shoot dieback	X					X	X	
Elongated black lesions (cankers)	X					X	X	
Trunk dieback	X	X	X	X	X		X	
Dark brown wood discoloration	X	X	X	X	X		X	Pascoe, 1998; Phillips, 2000; Castillo-Pando <i>et al.</i> , 2001; Phillips 2002
Brown streaking, black spots	X	X	X	X	X		X	
Bleached canes	X	X	X	X	X		X	
V-shaped necrotic sector	X	X	X	X	X	X	X	

Continued

Table 1. Continued

Symptoms	<i>B. dothidea</i>	<i>B. parva</i>	<i>B. obtusa</i>	<i>B. stevensii</i>	<i>B. lutea</i>	<i>B. ribis</i>	<i>B. rhodina</i>	Reference
Graft union failure	X	X	X	X	X		X	Pascoe, 1998; Phillips, 2000; Castillo-Pando <i>et al.</i> , 2001; Phillips, 2002
Leaf chlorosis				X		X		Lehoczy, 1974, 1988; Larignon & Dubos, 2001
								Milholland, 1988, 1991;
Fruit rot	X					X	X	Kummuang <i>et al.</i> , 1996a, 1996b

2. SYSTEMATIC REAPPRAISAL OF *CONIELLA* AND *PILIDIELLA*, WITH SPECIFIC REFERENCE TO SPECIES OCCURRING ON *EUCALYPTUS* AND *VITIS* IN SOUTH AFRICA

ABSTRACT

The genus *Pilidiella*, including its teleomorphs in *Schizoparme*, has a cosmopolitan distribution and is associated with disease symptoms on many plants. In the past, conidial pigmentation has been used as a character to separate *Pilidiella* (hyaline to pale brown conidia) from *Coniella* (dark brown conidia). In recent years, however, the two genera have been regarded as synonymous, with the older name, *Coniella*, having priority. To address the generic question, sequences of the internal transcribed spacer region (ITS 1, ITS 2), 5.8S gene, large subunit (LSU) and elongation factor 1- α gene (EF 1- α) were analysed to compare the type species of *Pilidiella* and *Coniella*. All three gene regions supported the separation of *Coniella* from *Pilidiella*, with the majority of taxa residing in *Pilidiella*. *Pilidiella* is characterised by having species with hyaline to pale brown conidia (avg. length:width >1.5), in contrast to the dark brown conidia of *Coniella* (avg. length:width ≤ 1.5). *Pilidiella diplodiella*, which is a pathogen associated with white rot of grapevines, was shown to be an older name for *C. petrakii*. To delineate species in the *P. diplodiella* species complex, isolates were also compared based on histone (H3) gene sequences. Analyses derived from these sequence data separated *P. diplodiella* from a newly described species, *P. diplodiopsis*. A new species, *P. eucalyptorum*, is proposed for the isolates formerly treated as *C. fragariae* and associated with leaf spots of *Eucalyptus* spp. This species clustered basal to *Pilidiella*, and may represent yet a third genus within this complex. *Pilidiella destruens* is newly described as anamorph of *Schizoparme destruens*, which is associated with twig dieback of *Eucalyptus* spp. in Hawaii. A key based on morphological characteristics is provided to separate the taxa treated in this study.

INTRODUCTION

The anamorph genera *Coniella* and *Pilidiella* have a cosmopolitan distribution and include plant pathogens that cause leaf, stem and root diseases on a wide variety of hosts. *Pilidiella* has been linked to teleomorphs in *Schizoparme* (Maas *et al.*, 1979). Van der Aa (in

von Arx, 1973) and von Arx (1981) treated *Coniella* and *Pilidiella* as separate genera with *Coniella* having dark brown conidia and *Pilidiella* hyaline to medium brown conidia. Sutton (1980) and Nag Raj (1993), however, treated the two genera as synonyms. Samuels *et al.* (1993) linked several *Coniella* anamorphs to species of *Schizoparme*, which the authors regarded as a member of the *Diaporthales* (*Melanconidaceae*). Recent DNA-based studies suggest that the *Schizoparme*-complex is representative of an undescribed family, and that the anamorph genera, *Coniella* and *Pilidiella*, should be retained as separate entities in the *Diaporthales* (Castlebury *et al.*, 2002).

Schizoparme was originally described for a single species, *Schizoparme straminea*, which was found on a wide variety of woody and herbaceous hosts (Shear, 1923). In 1979, the anamorph-teleomorph relationship for *S. straminea* was established when Maas *et al.* (1979) recognised that *Pilidiella castaneicola* (as *P. quercicola*), a hitherto unknown coelomycete isolated from strawberry, was the anamorph of *S. straminea*.

The most important species belonging to the *Coniella/Pilidiella* complex on grapevines is *C. diplodiella*, the causal agent of white rot (Sutton & Waterston, 1966). This disease is especially severe in cases where hailstorms have damaged vineyards, and many wounds are available for infection. Severe infections can reportedly lead to losses of between 20–80% (Bisiach, 1988). Sutton (1980) was unable to distinguish *C. diplodiella* from *C. fragariae*, a species that is known to commonly occur in soil, and also to cause leaf diseases of strawberries (Jarvis & Hargreaves, 1972) and *Eucalyptus* (Sharma *et al.*, 1985).

Coniella diplodiella and *C. fragariae* have previously been reported from South Africa (Crous *et al.*, 2000). Of these fungi, *C. diplodiella* is listed as an organism of quarantine importance. During 2000 several shipments of grapevine cuttings imported into South Africa from Europe and Australia were found to be contaminated with *C. diplodiella*, leading to their rejection. For this reason, Winetech, the body funding grapevine research in South Africa, requested a clarification of records of *Coniella* species from South Africa. The aims of this study were therefore to clarify the status of *C. diplodiella*, to compare it with other species in the genus, and to determine whether this species occurs in South Africa.

MATERIALS AND METHODS

DNA isolation, amplification and phylogeny

Thirty-four isolates of *Coniella* from South Africa, Europe, North and South America, Asia and Australasia were studied (Table 1). The methods of Lee and Taylor (1990) were used to isolate genomic DNA from fungal mycelium grown on potato dextrose agar (PDA; Biolab, Midrand, South Africa) plates.

The primers ITS1 and ITS4 (White *et al.*, 1990) were used to amplify part of the nuclear rRNA operon spanning the 3' end of the 18S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5' end of the 28S (large subunit) of the rRNA gene. The PCR reaction mixture consisted of 1.5 units Biotaq (Bioline, London, UK), 1x PCR buffer, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 4 pmoles of each primer, approximately 10 to 30 ng of fungal genomic DNA and was made up to a total volume of 25 µL with sterile water. Reactions were performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) and the cycling conditions comprised denaturation for 5 min at 96°C, followed by 30 cycles at 96°C (30 s), 55°C (30 s), 72°C (90 s) and a final 7 min extension step at 72°C to complete the reaction. Part of the elongation factor 1-alpha (EF-1α) gene was amplified with primers EF1-728F and EF1-986R (Carbone & Kohn, 1999) for a selected subset of representative isolates. Part of the histone 3 (H3) gene was amplified with primers H3-1a and H3-1b (Glass & Donaldson, 1995). PCR conditions were the same for EF-1α and H3 as for ITS, except for the MgCl₂ concentration, which was lowered to 1.5 mM for elongation factor and 2.0 mM for histone, respectively. The 5' end of the large subunit RNA (28S) (LSU) gene was amplified using primers LR0R (Rehner & Samuels, 1994) and LR7 (Vilgalys & Hester, 1990) using the same conditions as described here but with 4 mM MgCl₂. PCR products were separated by electrophoresis at 80 V for 1 h in a 0.8% (w/v) agarose gel in 0.5x TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining.

The amplification products were purified using NucleoSpin® Extract 2 in 1 kit (Macherey-Nagel, Germany). The purified products were sequenced in both directions using the PCR primers and the cycle sequencing reaction was carried out as recommended by the manufacturer with an ABI PRISM Big Dye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (Applied

Biosystems, Foster City, CA) containing AmpliTaq DNA Polymerase. The reaction was set up as denaturing at 94°C for 5 min, followed by 25 cycles of 96°C for 10 s, 55°C for 10 s, and 60°C for 4 min, with a final incubation of 30 s at 60°C. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

The ITS nucleotide sequences generated in this study were added to other ITS sequences obtained from GenBank (<http://www.ncbi.nlm.nih.gov>). The large subunit sequences were added to sequences obtained from the alignment of Castlebury *et al.* (2002). The alignments were assembled using Sequence Alignment Editor v2.0a11 (Rambaut, 2002) and manual adjustments for improvement were made visually where necessary. The phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis & Bull, 1993). Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated. The resulting trees were printed with TreeView Version 1.6.6 (Page, 1996). A partition homogeneity test (Farris *et al.*, 1994) was conducted in PAUP (Swofford, 2000) to consider the feasibility of combining the various sequence data sets.

Morphology

Cultures were grown on PDA and placed under mixed cool white fluorescent and nuv light at 25°C to enhance sporulation. Morphological observations were made from structures mounted in lactic acid. The 95% confidence intervals of conidial measurements were derived from at least 30 observations at x 1000 magnification. As certain species show overlapping conidial dimensions, but differ regarding spore volume, the average conidial length:width (l:w) is provided to further distinguish these taxa (Nag Raj, 1993). Growth rates, cultural characteristics and cardinal temperature requirements for growth were determined for isolates plated onto PDA in 90 mm Petri dishes and incubated in the dark for 7 days at seven different temperatures, ranging from 5°C to 35°C in 5°C intervals. Three plates were used for each isolate at each temperature. Radial mycelial growth was assessed using two perpendicular measurements for each plate, and the means calculated to determine the growth rates for each

species. The colours of cultures were described from isolates incubated at 25°C in the dark for 2 wks using the colour charts of Rayner (1970). Isolates are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (STE-U), and the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (CBS).

RESULTS

Phylogenetic analyses

Partition homogeneity tests (where $P \geq 0.05$ was taken as significantly incongruent) of the different datasets indicated that they were combinable. However, since different questions at generic and species level are addressed using different datasets, the phylogenetic data are presented as separate rather than combined trees. The large subunit alignment was used for inference of the higher order taxonomic relationship between *Pilidiella* and *Coniella*, while the ITS alignment was used to discriminate species and species complexes. Species relationships between *P. diplodiella*, *P. diplodiopsis*, *P. eucalyptorum* and *C. fragariae* were established with the elongation factor 1- α alignment. The division between *P. diplodiella* and *P. diplodiopsis* was investigated further and confirmed using alignment of the histone gene sequences. New sequences were deposited in GenBank (Table 1), and the alignments in TreeBASE (SN 1525).

The large subunit sequence alignment contained 25 taxa and spanned 1255 characters, including the gaps (in TreeBASE). Of the aligned nucleotide sites for the data set, 138 characters were parsimony-informative, six variable characters were parsimony-uninformative and 1111 were constant. Maximum parsimony analysis of the sequence data resulted in 47 equally most parsimonious trees (TL = 178 steps, CI = 0.848, RI = 0.904, RC = 0.767), one of which is shown in Fig. 1. Most of the isolates grouped with the type strain of *Schizoparme straminea* (CBS 149.22) (58% support). The *P. diplodiella/diplodiopsis* isolates grouped together (56% bootstrap support) and three of these isolates (CBS 111857, 111858, 166.84) formed a separate cluster (60% bootstrap support) within this group. Two isolates of *Pilidiella* occurring on *Eucalyptus* (STE-U 3905 and CBS 112640) formed a well-supported cluster (100% support) within the *Pilidiella* clade, as did isolates of *P. granati* (97% support). The *C. fragariae* isolates formed a separate clade with a bootstrap support value of 100%.

The manually adjusted alignment of the ITS nucleotide sequences contained 36 taxa and 504 characters including alignment gaps (in TreeBASE). Of the aligned nucleotide sites, 90

characters were parsimony-informative, 37 variable characters were parsimony-uninformative and 377 were constant. Six equally most parsimonious trees (TL = 192 steps, CI = 0.859, RI = 0.965, RC = 0.829) were obtained from maximum parsimony analysis of the ITS sequence data, one of which is shown (Fig. 2). The *Pilidiella* isolates sequenced in this study grouped in a single *Pilidiella* clade (100% bootstrap support), with two well-supported subclades. The first subclade (92% bootstrap support), contained two isolates of *P. castaneicola*, an isolate of *P. granati*, *S. straminea*, unnamed *Pilidiella* spp., and isolates initially identified as *P. diplodiella* and *P. petrakii*. The correct names for the *P. diplodiella* and *P. petrakii* isolates are shown in the tree. All the isolates from symptomatic *Eucalyptus* leaves formed another well-supported subclade (100% bootstrap support) basal to the *Pilidiella* subclade. A further clade (bootstrap support of 100%) contained *Coniella fragariae* isolates from various countries and hosts, as well as an isolate of *C. australiensis*.

Part of the elongation factor 1- α gene was sequenced for a subset of isolates. The manually adjusted alignment of the nucleotide sequences contained eighteen taxa and 452 characters including alignment gaps (in TreeBASE). Of the aligned nucleotide sites 304 characters were parsimony-informative, 60 variable characters were parsimony-uninformative and 88 were constant. The elongation factor 1- α sequence data were also subjected to maximum parsimony analysis and resulted in a single most parsimonious tree (TL = 1056 steps, CI = 0.701, RI = 0.785, RC = 0.550). The phylogenetic tree (Fig. 3) delimited several clades that correlated with the ITS and LSU trees. As with the ITS and LSU trees, the clade containing the *Pilidiella* isolates (56% bootstrap support) contained two subclades. The first subclade (99% bootstrap support) contained *P. macrospora*, *P. granati*, a cluster (88% bootstrap support) containing the isolates *S. straminea* and *P. castaneicola* and a cluster (97% bootstrap support) containing an isolate of *P. castaneicola* and isolates originally identified as *P. diplodiella*. In the *P. diplodiella* cluster (98% bootstrap support), isolates were further divided into two clusters (100% bootstrap support, respectively) containing *P. diplodiella* and a previously undescribed species, *P. diplodiopsis*. Isolates from *Eucalyptus* grouped in a second (100% bootstrap support) subclade basal to *Pilidiella* while those of *C. fragariae* formed a separate well-supported cluster (100% bootstrap support).

To further evaluate the subdivision within the *P. diplodiella* isolates, approximately 500 bases of the histone gene were sequenced for the isolates and the manually adjusted alignment of the nucleotide sequences contained sixteen taxa and 505 characters including alignment gaps (in TreeBASE). Of the aligned nucleotide sites for the data set, 309 characters were parsimony-informative, 37 variable characters were parsimony-uninformative, and 159 were constant. Maximum parsimony analysis of the histone sequence data resulted in a single most parsimonious

tree (Fig. 4; TL = 462 steps, CI = 0.918, RI = 0.919, RC = 0.844). As with the elongation factor data, the isolates previously identified as *P. diplodiella* formed a well-supported clade (96 % bootstrap support), which was further divided into two major clusters (each with 100 % bootstrap support) containing isolates of *P. diplodiella* and *P. diplodiopsis*. A further species of *Pilidiella* (IMI 100482) formed a poorly supported group (67 % bootstrap support value), with the *P. diplodiella* isolates. Isolate CBS 111021, which also represented a distinct species of *Pilidiella*, was placed outside the clade containing isolates of *P. diplodiella* and *P. diplodiopsis*.

Morphology

***Coniella australiensis* Petrak, Sydowia 9, 567 (1955).**

Figs. 13–14

Pycnidia globose, 120–200 µm wide, initially appearing hyaline with a dark brown, internal conidial mass, becoming brown with age; ostiole central, 10–15 µm wide; wall 10–20 µm thick, consisting of 3–4 layers of medium brown *textura angularis*; pycnidia containing a basal, central cushion of hyaline cells that give rise to hyaline conidiophores. *Conidiophores* densely aggregated, slender, subulate, simple, frequently branched above, reduced to conidiogenous cells, or with 1–2 supporting cells, 12–25 x 4–5 µm. *Conidiogenous cells* simple, tapering, hyaline, smooth, 7–15 x 3–4 µm, 1.5–3 µm wide at apex, surrounded by a gelatinous coating, apex with visible periclinal thickening. *Conidia* broadly ellipsoidal, (9–)10–11(–14) x (6–)7–8(–10) µm, (l:w = 1.4), apices obtuse, base subtruncate, inequilateral, multiguttulate when young, bi-guttulate when mature, hyaline to pale brown, becoming dark brown at maturity, wall of medium thickness, smooth, germ slits absent; small, hyaline, mucoid basal appendage frequently present, 1–2 µm in length. Description based on IMI 334797 *in vitro*.

Cultures. Colonies raised, olivaceous buff (21''d) on the surface, and greenish olivaceous (23''i) underneath, reaching 39 mm after 7 d at 25°C. Cardinal temperature requirements for growth: min. 5°C, max. 30°C, opt. 25°C.

Specimens examined. **South Africa:** *Gauteng*: on leaf litter, 1981, K.T. van Warmelo, IMI 261318 = STE-U 4196; *Western Cape Province*: Cape Town, Kirstenbosch Botanical Gardens, on *Pelargonium centrilobum*, 11 Aug. 1989, J. Isaacs, PREM 50600, culture IMI 334797.

Notes. The conidial shape and absence of a germ slit distinguishes this species from *C. fragariae*.

***Coniella fragariae* (Oudem.) B. Sutton, *Mycol. Pap.* 141, 47 (1977).**

Figs. 5–12

Basionym: *Coniothyrium fragariae* Oudem., *Verh. Kon. Ned. Akad. Wetensch. ser. 2*, 18: 37 (1883).

Synonyms listed in Sutton (1980).

Pycnidia globose to depressed, 250–500 µm wide, initially appearing hyaline with a dark brown, internal conidial mass, becoming brown with age; ostiole central, 10–50 µm wide; wall 20–30 µm thick, consisting of 3–6 layers of pale to medium brown *textura angularis*; pycnidia containing a basal, central cushion of hyaline cells that give rise to hyaline conidiophores. *Conidiophores* densely aggregated, slender, subulate, simple, frequently branched above, reduced to conidiogenous cells, or with 1–2 supporting cells, 15–30 x (2–)3–4 µm. *Conidiogenous cells* simple, tapering, hyaline, smooth, 10–20 x 2–3 µm, 1–1.5 µm wide at apex, surrounded by a gelatinous coating, apex with visible periclinal thickening, rarely with percurrent proliferation. *Conidia* ellipsoidal, apices tapering, narrowly obtusely rounded, tapering from middle towards a narrowly subtruncate base, medium brown, multiguttulate when young, mostly 1–2-guttulate when mature, wall of medium thickness, darker brown than medium brown body of conidium, frequently with a lighter band of pigment extending over conidium, with a germ slit visible in older conidia, and mucous appendages also visible in lactophenol; appendages mostly basal, but also lateral along the length of the conidium, (8–)9–10(–12.5) x (5–)6–7(–8) µm (l:w = 1.5). *Microconidia* also observed in some cultures, cylindrical, hyaline, straight with obtuse ends, 4–5 x 1–1.5 µm.

Cultures. Colonies flat, white on the surface, and pale luteous (17 f) in reverse, reaching 32 mm after 7 d at 25°C. Cardinal temperature requirements for growth: min. 5°C, max. 35°C, opt. 30°C.

Specimens examined. **South Africa:** *Western Cape Province:* Paarl, Bienne Donne, on *Fragaria* sp., C. Roux, 8 Dec. 1986, PREM 48853 (pycnidia of *Septoria aciculosa* and *Gnomonia comari* are also present; both are new records for South Africa); *Mpumalanga:* Sabie, Tweefontein nursery, *Pinus elliotii* seedlings, N.J. Van Rensburg, 19 Sept. 1986, PREM 48889 = IMI 312146; *Eastern Cape Province:* East London, on roots of *Ananas* sp., M. Dallsdorf, Feb. 1968, PREM 44310.

Notes. Although analysis of sequence data did not distinguish *C. australiensis* from *C. fragariae*, the two species have distinct conidial shapes, with those of *C. australiensis* being wider, more broadly ellipsoidal, (6–)7–8(–10) µm, and having more obtusely rounded apices, than the more tapering apices of *C. fragariae*. *C. fragariae* is also characterized by forming copious

amounts of mucous that encase the conidiophores. This forms a mucous sheath through which subsequent conidia extend. At dehiscence, the remains of this mucus are frequently visible as a basal conidial appendage, while in rare cases the sheath encases the whole conidium, and this can be seen as an appendage extending at either end of the conidium. This species was reported as *C. pulchella* by Marasas & Van der Westhuizen (1971). The latter name has been reduced to synonymy with *C. fragariae* (Sutton 1980). The specimen of the South African record (PREM 44310) was examined, and confirmed to be the same as *C. fragariae*.

***Pilidiella diplodiella* (Speg.) Crous & J.M. van Niekerk, comb. nov.** Figs. 15–17, 22–23

Basionym: *Phoma diplodiella* Speg., *Ampelomiceti Italici* No. 4 (1878).

Coniothyrium diplodiella (Speg.) Sacc., *Syll. Fung.* **3**, 310 (1884).

Coniella diplodiella (Speg.) Petr. & Syd., *Feddes Repert. Beih.* **42**, 460 (1927).

Coniella petrakii B. Sutton, in Sutton, *The Coelomycetes, Fungi Imperfecti with Pycnidia, Acervuli and Stromata*: 422 (1980).

Pycnidia globose and slightly depressed to subglobose, in some cases tapering slightly towards the ostiole, 200–350 µm wide, smooth, initially hyaline with a dark central conidial mass, becoming dark brown, ostiole central, up to 100 µm wide, with cells darker brown around the ostiole; wall 15–25 µm thick, consisting of 3–5 layers of medium brown *textura angularis*; pycnidia containing a basal, central cushion of hyaline cells that give rise to conidiophores. *Conidiophores* dense, slender, simple or branched below, 0–3-septate, 10–20 x 3–4 µm, surrounded by a mucous coating. *Conidiogenous cells* simple, slender, hyaline, smooth, 8–15 x 2–3 µm, 1 µm wide at the apex, with prominent periclinal thickening. *Conidia* hyaline when immature, becoming pale to medium brown, inequilateral, smooth, frequently with a hyaline, lateral appendage, narrowly ellipsoidal, apices tapering, subobtusely rounded, bases subtruncate, multiguttulate, straight to slightly curved, wall of medium thickness, multiguttulate, (10–)12–15(–19) x (4–)5–6 µm (l:w = 2.3).

Cultures. Colonies flat, buff (19''d) coloured on surface, and honey (21''b) in reverse, reaching 36 mm after 7 d at 25°C. Cardinal temperature requirements for growth: min. 5°C, max 35°C, opt. 30°C.

Specimens examined. **France:** on stems of *V. vinifera*, P.W. Crous, 2000, herb CBS 6948, EPITYPE of *Pilidiella diplodiella* designated here, culture ex-epitype, CBS 111858, STE-U 3708. **India:** on *Vitis vinifera*, M.A. Salam, 29 Jan. 1965, HOLOTYPE of *Pilidiella petrakii*, IMI 112027.

Italy: on seed of *Vitis vinifera*, *Briosi & Cavara No. 48*, PREM 7870; Conegliano, on *Vitis vinifera*, *C.L. Spegazzini*, 1877, HOLOTYPE of *Phoma diplodiella* at Univ. Nacional de la Plata, Museo, Inst. Spegazzini No. 11518 missing, slides ex-type WINF(M) 7526.

Notes. *Pilidiella diplodiella* (as *C. petrakii*) has previously been recorded from South Africa on *Eucalyptus* (Lundquist & Baxter, 1985). An examination of this specimen showed that it was not *C. petrakii*, but *C. petrakioidea*. *Pilidiella diplodiella* (as *Coniella*) was first reported from South Africa in 1977 by Verbeek in the Annual Report of the Secretary for Agricultural Services (Crous & Carstens, 2000). A second report of the fungus was published by Matthee & Thomas (1981). However, the morphology and cultural characteristics were not described and no herbarium specimens were lodged, making confirmation of these records impossible (Crous & Carstens, 2000). The present study has shown that *C. diplodiella* does occur on grapevines in South Africa, and that it should no longer be considered as an organism of quarantine significance.

On the type specimen of *Phoma diplodiella*, we found two fungi, namely *Pilidiella diplodiella* and *Coniothyrium olivaceum*. Sutton (1969) studied the same specimen, and described loose conidia on the surface similar to those of *P. diplodiella*, i.e. “medium brown, ellipsoidal, having truncate bases and smooth walls, 10–10.5 x 5 µm”. As the type specimen of this fungus consists of two slide preparations that are in poor condition, Sutton (1969) regarded *Phoma diplodiella* as a *nomen dubium*. In our examination of the same specimen, conidia appeared thick-walled and finely verruculose. Conidia were 9–15 x 4–6 µm, ellipsoidal with subobtuse to obtusely rounded apices, thus fitting the general description of *P. diplodiella*. Sutton (1980) described a similar species, *C. petrakii*, which was later reduced to synonymy with *C. diplodiella* (IMI Distribution Map No. 335, Ed. 3, 1992). After examination of type material of both fungi, we agree that they are synonymous, and that the older name should be used for this fungus. Given the fact that conidia are initially hyaline, becoming pale brown, this species is assigned to *Pilidiella*, and a new epitype specimen and culture are designated.

***Pilidiella diplodiopsis* Crous & J.M. van Niekerk, sp. nov.**

Figs. 25–26

Pilidiellae diplodiellae similis, sed conidiis anguste ellipsoideis, sursum magis angustatis distincta; conidia breviora, (8–)10–12(–13) x (5–)6–7(–7.5) µm.

HOLOTYPE: **Italy:** on canes of *Vitis vinifera*, herb CBS 6947, ex-type culture CBS 590.84.

Pycnidia globose and slightly depressed to subglobose, in some cases tapering slightly towards the ostiole, 200–400 µm wide, smooth, initially hyaline with a dark central conidial mass,

becoming dark brown, ostiole central, up to 150 μm wide; wall 10–25 μm thick, consisting of 3–6 layers of medium brown *textura angularis*; pycnidia containing a basal, central cushion of hyaline cells that give rise to conidiophores. *Conidiophores* dense, slender, simple or branched below, 0–3-septate, 10–35 x 3–4 μm , surrounded by a mucous coating. *Conidiogenous cells* simple, slender, hyaline, smooth, 10–15 x 2–3 μm , 1 μm wide at the apex, with prominent periclinal thickening. *Conidia* pale to medium brown, narrowly ellipsoidal with attenuating conidial apices that are acutely rounded, (8–)10–12(–13) x (5–)6–7(–7.5) μm (l:w = 1.7).

Cultures. Similar to that described for *P. diplodiella*.

Specimen examined. **Italy:** on canes of *Vitis vinifera*, HOLOTYPE in herb CBS 6947, ex-type culture CBS 590.84, STE-U 3940.

Notes. Morphologically similar to *P. diplodiella*, but distinct in having conidia that are pale to medium brown, narrowly ellipsoidal, but with more attenuating conidial apices (less pronounced when mature), that are acutely rounded; conidia also shorter, (8–)10–12(–13) x (5–)6–7(–7.5) μm , with a lower l:w (1.7). Presently *P. diplodiopsis* is known from grapevines in Italy and Switzerland (Table 1).

***Pilidiella eucalyptorum* Crous & M.J. Wingf., sp. nov.**

Figs. 18–21, 27

Coniellae fragariae similis, sed conidiis maioribus, (9–)10–12(–14) x (6–)7–8 μm , late ellipsoideis vel limoniformibus, sursum acute rotundatis distincta.

HOLOTYPE: **Australia:** on leaves of *Eucalyptus* sp., *P.Q. Thu & R.J. Gibbs*, in herb CBS 6946, ex-type culture CBS 112640.

Leaf spots large, irregular, pale brown with diffuse margins, frequently secondary, associated with primary pathogens or with insect or wind damage. *Pycnidia* subepidermal, erumpent, exuding masses of black conidia that disperse with water over the leaf surface; pycnidia frequently forming in concentric circles from point of infection; pycnidia in culture with a red brown ostiolar area and base; pycnidia globose, up to 300 μm wide, smooth, medium to dark brown, with a central ostiole, up to 60 μm wide; wall up to 25 μm wide, 3–5 layers of medium brown *textura angularis*; pycnidia containing a central basal cushion of hyaline cells that give rise to conidiophores. *Conidiophores* densely aggregated, hyaline, smooth, slender, branched, 1–3-septate, 15–25 x 4–5 μm ; conidiophores similar to those of *C. fragariae*, but surrounded by less mucous, and more branched than in *C. fragariae*. *Conidiogenous cells* hyaline, smooth, with prominent periclinal thickening at the apex, rarely proliferating percurrently, 10–17 x 3–3.5 μm .

Conidia medium to dark red–brown, broadly ellipsoidal or limoniform, widest in the middle, tapering to an acutely rounded apex and a subtruncate base, multiguttulate, with a longitudinal germ slit, wall of medium thickness as in *C. fragariae*, but basal mucoid appendage less common than in *C. fragariae*, (9–)10–12(–14) x (6–)7–8 µm (l:w = 1.6).

Cultures. Colonies olivaceous (21''i) on the surface and citrine green (23''b) in reverse, reaching 20 mm after 7 d at 25 °C. Cardinal temperature requirements for growth: min. 10 °C, max. 30 °C, opt. 30 °C.

Specimen examined. Australia: on leaves of *Eucalyptus* sp., *P.Q. Thu & R.J. Gibbs*, HOLOTYPE in herb CBS 6946, ex-type culture CBS 112640.

Notes. This species has been regarded as *C. fragariae* (Sharma *et al.*, 1985, Park *et al.*, 2000), and is similar to it. It shares the same dark conidial pigmentation, and also has the same germ slits found in *C. fragariae*. Conidia of *P. eucalyptorum* are slightly larger, (9–)10–12(–14) x (6–)7–8 µm, than those of *C. fragariae*, (8–)9–10(–12.5) x (5–)6–7(–8) µm. The most obvious difference, however, lies in their conidial shape and colour. Conidia of *P. eucalyptorum* have acutely rounded apices, are red–brown, and frequently limoniform. In contrast those of *C. fragariae* are brown, and have tapering, narrow, obtusely rounded apices, and are never limoniform in shape.

***Pilidiella granati* (Sacc.) Aa, *Verh. Kon. Ned. Akad. Wetensch. Afd. Natuurk. Ser. 2* 61, 51 (1972).**

Fig. 29

Basionym: *Phoma granati* Sacc., *Nuovo G. Bot. Ital.* 8, 200 (1876).

For synonyms and description see Nag Raj (1993).

Cultures. Colonies straw coloured (21'd) on the surface, and pale luteous (17 f) in reverse, reaching 28 mm after 7 d at 25°C. Cardinal temperature requirements for growth: min. 20°C, max. 30°C, opt. 30°C.

Specimen examined. South Africa: Gauteng: Pretoria, *Fragaria* sp., *G.C.A. Van der Westhuizen & K.T. Van Warmelo*, 1978, IMI 233050.

Notes. Conidia of IMI 233050 were fusiform, 15–27 x 2.5–3.5 µm, thus longer than those of *C. granati* (CBS 252.38), and the description provided for this species by Nag Raj (1993). This suggests that the collection represents *C. castaneicola*, and that the record of *C. granati* from South Africa is incorrect.

***Pilidiella petrakioidea* (Nag Raj) Crous & J.M. van Niekerk, comb. nov.** Figs 24, 33–34

Basionym: *Coniella petrakioidea* Nag Raj, in Nag Raj, *Coelomycetous Anamorphs with Appendage-bearing Conidia* (Ontario): 233 (1993).

For synonyms and description see Nag Raj (1993).

Specimen examined. **South Africa:** on *Eucalyptus* sp., J. Lundquist, 22 Jun. 1982, PREM 47146.

Notes. Conidia are pale brown to brown, (9–)10–12(–15) x 7–8 µm, and narrowly ellipsoidal with acutely rounded apices and a lateral mucous sheath, thus closely matching the description of *C. petrakioidea* provided by Nag Raj (1993). The hyaline to pale brown conidia, suggest that this species is more appropriately accommodated in *Pilidiella* than in *Coniella*. No cultures of *P. petrakioidea* were available for study.

***Schizoparme destruens* (M.E. Barr & Hodges) Samuels, M.E. Barr & Lowen, Mycotaxon 46: 470 (1993).** Figs. 30–32, 35–41

Anamorph: ***Pilidiella destruens*** Crous & M.J. Wingf., **sp. nov.**

Pilidiellae diplodiellae similis, sed conidiis fusoideo-ellipsoideis, (10–)12–13(–15) x (3–)4–5(–6) µm (long.:lat. 2.7), sursum paene obtuse rotundatis distincta.

HOLOTYPE: **USA:** *Hawaii:* on twigs of *Eucalyptus grandis*, M.J. Wingfield, Oct. 2000, herb CBS 6945.

Perithecia caulicolous, solitary, subepidermal, becoming erumpent, but not superficial as described for the type (Samuels *et al.*, 1993), globose, up to 300 µm wide, apex short papillate, dark brown; wall up to 80 µm wide, consisting of three regions, namely an outer warty region visible near erumpent apical part of perithecium, 20–40 µm wide; an intermediate layer of medium brown *textura angularis*, 15–20 µm wide. an inner layer of thin-walled, flattened, hyaline cells, 5–15 µm; ostiolum central, circular, up to 75 µm wide; ostiolar chanal lined with slender, septate, hyaline, thin-walled periphysoids, 15–20 x 2–3 µm. *Asci* clavate, 35–50 x 7–14 µm; with inconspicuous apical apparatus, 8-spored, bi-seriate. *Ascospores* ellipsoidal, hyaline, thick-walled, granular, with terminal mucous caps, (9–)11–13 x (4.5–)5–6 µm.

Pycnidia intermingled between perithecia, globose, up to 200 µm wide; ostiole central, consisting of dark brown cells, up to 50 µm wide; wall consisting of 3–5 layers of medium brown

textura angularis; pycnidia containing a basal, central cushion of hyaline cells that give rise to conidiophores. Conidiophores dense, slender, branched, hyaline, smooth, 15–25 x 3–5 µm. Conidiogenous cells slender, tapering towards a truncate apex, smooth, hyaline, 10–15 x 2–3 µm, 1 µm wide at the apex, with minute periclinal thickening, coated in mucous. Conidia long, fusoid–ellipsoidal, widest in the middle, tapering to an acutely rounded apex and subtruncate base with minute scar, pale to medium brown, granular, wall of medium thickness, (10–)12–13(–15) x (3–)4–5(–6) µm (l:w = 2.7).

Specimen examined. USA: Hawaii: on twigs of *Eucalyptus grandis*, M.J. Wingfield, Oct. 2000, HOLOTYPE of anamorph, herb CBS 6945 (cultures none).

Notes. The morphology of the teleomorph in our collection closely matches that of the type specimen, which is known from *Eucalyptus globulus* twigs collected in Hawaii (Samuels *et al.*, 1993). Although the anamorph-teleomorph connection could not be verified in culture, pycnidia of the *Pilidiella* anamorph occurs intermingled with perithecia of the *Schizoparme* teleomorph. The conidia of *P. diplodiella* and *P. destruens* are similar, but differ in shape. Conidia of *P. diplodiella* have a l:w of 2.3, and acutely rounded apices, whereas those of *P. destruens* have l:w of 2.7, and subobtusely rounded apices.

***Schizoparme straminea* Shear, *Mycologia* 15, 121 (1923).**

Fig. 28

Anamorph: *Pilidiella castaneicola* (Ellis & Everh.) Arx, *Verh. Kon. Ned. Akad. Wetensch. Afd. Natuurk.* 51, 69 (1957).

Basionym: *Gloeosporium castaneicola* Ellis & Everh., *Proc. Acad. Nat. Sci. Philadelphia*: 435 (1895).

Coniella castaneicola (Ellis & Everh.) B. Sutton, in Sutton, *The Coelomycetes, Fungi Imperfecti with Pycnidia, Acervuli and Stromata*: 420 (1980).

For synonyms and description see Nag Raj (1993).

debris, 1989, *E. van der Linde*, PREM 50631, culture IMI 334801; Tzaneen, on leaves of *Eucalyptus globulus* seedlings, 27 Mar. 1991, *P.W. Crous*, PREM 51101; *Mpumalanga*: Barberton, on leaves of *Eucalyptus* sp., 27 Mar. 1991, *P.W. Crous*, PREM 51098.

Notes. This species is commonly encountered on *Eucalyptus* leaves, but is generally regarded to be of minor importance as a foliar pathogen. Morphologically, it is most similar to *P. granati*, although the two fungi can easily be distinguished. *P. quercicola* has fusiform to naviculate conidia which are longer and narrower (13–29 x 2.5–4 µm), than the ellipsoidal conidia of *P. granati* (9–16 x 3–4.5 µm) (Nag Raj, 1993).

DISCUSSION

Conidial pigmentation has been used to separate *Pilidiella* from *Coniella* (von Arx, 1981), but was rejected as a distinguishing characteristic by Sutton (1980) and Nag Raj (1993) who used the older name, *Coniella*. Results of a study by Castlebury *et al.* (2002) suggested, however, that *Pilidiella* with its teleomorphs in *Schizoparme* represents a genus distinct from *Coniella*. In the present study, additional species were examined, and analysed based on their ITS, EF 1- α and LSU sequence data. All three data sets confirmed the separation of *Pilidiella* typified by *P. castaneicola* from *Coniella* typified by *C. fragariae* (Figs 1–3). *Pilidiella* is characterised by having species with hyaline to pale brown conidia (l:w >1.5), in contrast to the dark brown conidia of *Coniella* (l:w \leq 1.5).

Until now, isolates from *Eucalyptus*, herein recognised as *P. eucalyptorum*, have been treated as representative of *C. fragariae* (Sharma *et al.*, 1985; Park *et al.*, 2000). Other than conidial shape, length:width and colour, *P. eucalyptorum* is similar to *C. fragariae*. Based on its dark conidia, this species should be classified in *Coniella sensu* von Arx (1981). However, *P. eucalyptorum* clustered basal to the distinct *Pilidiella* clade in the LSU, ITS and EF 1- α analyses (Figs 1–3). Although treated as a species of *Pilidiella*, the possibility exists that *P. eucalyptorum* may represent yet a third discrete genus within this complex.

The link between *Schizoparme* and *Pilidiella* has been reconfirmed in this study. Other than reporting a *Pilidiella* anamorph for *S. destruens*, a possible link is also shown between *P. macrospora* and *S. botrytidis* (Fig. 1).

Although *C. australiensis* is distinguishable from *C. fragariae* based on morphology, these differences were not supported by analyses of LSU and ITS sequence data. Isolates of *C.*

fragariae have commonly been obtained from soil, but were also associated with disease symptoms of *Fragaria* and *Vitis*. Isolate CBS 111021, from *Fragaria* in South Africa, clustered within *Pilidiella* based on ITS data (Fig. 2), and separated from it based on the histone sequence data (Fig. 4), which provided a better separation of closely related taxa than the ITS sequences. Morphologically, this isolate is distinct from *C. fragariae*, in having more ellipsoidal to limoniform conidia, (10–)11–13(–15) x 6–7(–7.5) μm (l:w = 1.8), with acutely rounded apices, and probably represents an undescribed species.

The taxonomic position of isolates residing in the *P. petrakii/diplodiella* complex on grapevines cannot be resolved based on these data. A re-examination of type material revealed that '*C.*' *diplodiella* is the older name for the fungus treated as '*C.*' *petrakii* (Sutton 1980). The type specimens of these two species closely resemble the morphology of isolates clustering in the main clade of *P. diplodiella* (Fig. 2), which represents isolates collected from grapevines in Australia, France, Germany, India, Italy, Switzerland and South Africa. Analyses of sequences of the elongation factor 1- α , histone and the LSU regions for a subset of isolates (Figs 1, 3 and 4), suggest that the *P. diplodiella* complex contains two species, *P. diplodiella* (with *C. petrakii* as synonym), *P. diplodiopsis*, as well as some undescribed species. Presently *P. diplodiopsis* is known from isolates collected in Switzerland and Italy. The isolate collected on *Vitis* in India (IMI 100482) is distinct, as its conidia are pale brown, and more narrowly ellipsoidal with acutely rounded apices, (9–)10–12(–15) x 4–5 μm .

Artificial inoculations on grapevines by von Tiedeman (1985) showed that isolates of *P. diplodiella* (as *C. petrakii*), and to a lesser extent *C. fragariae*, could both cause white rot symptoms of grapevines. Our data suggest that both these species are widely distributed, and that *P. diplodiella* may occur in most countries where grapevines are cultivated.

Key to selected species

- 1 Conidia consistently hyaline, or with a greenish tinge..... 2
- 1 Conidia pale to dark brown when mature 3
- 2 Conidia ellipsoidal, 9–16 μm long (l:w = 1.9) *Pilidiella granati*
- 2 Conidia fusiform to falcate or naviculate, 13–29 μm long (l:w = 7.1)
..... *Schizoparme straminea* (*Pilidiella castaneicola*)
- 3 Conidial broadly ellipsoidal with obtuse apices, germ slits absent, (9–)10–11(–14)
x (6–)7–8(–10) μm , (l:w = 1.4) *Coniella australiensis*

- 3 Conidia ellipsoidal, but tapering towards apices (l:w > 1.4) 4
- 4 Conidial mean l:w > 2 5
- 4 Conidial mean l:w < 2 6
- 5 Conidia fusoid-ellipsoidal, apices acutely rounded, (10-)12-13(-15) x
(3-)4-5(-6) μm (l:w = 2.7)..... *Schizoparme destruens* (*Pilidiella destruens*)
- 5 Conidia narrowly ellipsoidal, apices subobtusely rounded, (10-)12-15(-19) x (4-)
5-6 μm (l:w = 2.3) *Pilidiella diplodiella*
- 6 Conidia with germ slits; broadly ellipsoidal or limoniform (l:w = 1.5-1.6) 7
- 6 Conidia without germ slits; narrowly ellipsoidal (l:w = 1.7-1.8) 8
- 7 Conidia ellipsoidal, apices narrowly obtusely rounded, (8-)9-10(-12.5) x
(5-)6-7(-8) μm (l:w = 1.5)..... *Coniella fragariae*
- 7 Conidia broadly ellipsoidal or limoniform, apices acutely rounded,
(9-)10-12(-14) x (6-)7-8 μm (l:w = 1.6) *Pilidiella eucalyptorum*
- 8 Conidia (8-)10-12(-13) x (5-)6-7(-7.5) μm (l:w = 1.7)... *Pilidiella diplodiopsis*
- 8 Conidia (9-)10-12(-15) x 7-8 μm (l:w = 1.9)..... *Pilidiella petrakioidea*

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Table 1. *Coniella* and *Pilidiella* isolates studied

Species	Accession no. ¹	Substrate	Country	Collector	Areas sequenced ²			
					LSU	ITS	EF1- α	H3
<i>C. australiensis</i>	IMI 261318, CBS 111025, STE-U 4196	Leaf litter	South Africa	K. T. van Warmelo		AY339313		
<i>C. fragariae</i>	CBS 172.49, STE-U 3930	<i>Fragaria</i> sp.	Belgium	A. Jaarsveld	AY339282	AY339317	AY339352	
<i>C. fragariae</i>	IMI 111019, STE-U 3772	<i>Fragaria</i> sp.	South Africa	P. W. Crous		AY339316	AY339351	
<i>C. fragariae</i>	CBS 167.84, STE-U 3934	<i>V. vinifera</i>	Germany	A. von Tiedemann		AY339318		
<i>C. fragariae</i>	IMI 253210, STE-U 4199	Unknown	USA	B. C. Sutton	AY339283	AY339320		
<i>C. fragariae</i>	IMI 081599, CBS 165.60, STE-U 4198	Soil	India	P. N. Mathur <i>et al.</i>		AY339319		
<i>C. fragariae</i>	CBS 766.71, STE-U 3713	Unknown	South Africa	H. A. van der Aa		AY339315	AY339350	
<i>C. fragariae</i>	STE-U 3327	<i>Fragaria</i> sp.	South Africa	P. W. Crous		AY339314	AY339349	
<i>P. diplodiella</i>	CBS 166.84, STE-U 3931	<i>V. vinifera</i>	Germany	A. von Tiedemann	AY339286	AY339331	AY339357	AY339305
<i>P. diplodiella</i>	CBS 111858 ³ , STE-U 3708	<i>V. vinifera</i>	France	P. W. Crous	AY339284	AY339323	AY339355	AY339297

Continued

Table 1. Continued

Species	Accession no. ¹	Substrate	Country	Collector	Areas sequenced ²			
					LSU	ITS	EF1- α	H3
<i>P. diplodiella</i>	STE-U 3709	<i>V. vinifera</i>	Australia	P. W. Crous		AY339324		AY339298
<i>P. diplodiella</i>	CBS 111857	<i>V. vinifera</i>	South Africa	F. Halleen	AY339285	AY339325	AY339356	AY339299
	STE-U 3735							
<i>P. diplodiella</i>	CBS 111022,	<i>V. vinifera</i>	South Africa	F. Halleen		AY339326		AY339300
	STE-U 3736							
<i>P. diplodiella</i>	STE-U 3768	<i>V. vinifera</i>	France	P. W. Crous		AY339327		AY339301
<i>P. diplodiella</i>	STE-U 3769	<i>V. vinifera</i>	France	P. W. Crous		AY339328		AY339302
<i>P. diplodiella</i>	STE-U 3775	<i>V. vinifera</i>	France	P. W. Crous		AY339329		AY339303
<i>P. diplodiella</i>	STE-U 3778	<i>V. vinifera</i>	France	P. W. Crous		AY339330		AY339304
<i>P. diplodiopsis</i>	CBS 109.23,	<i>V. vinifera</i>	Switzerland	H. Faes	AY339287	AY339332	AY339358	AY339306
	STE-U 3933							
<i>P. diplodiopsis</i>	CBS 169.55,	<i>V. vinifera</i>	Switzerland	M. Staehelin		AY339333		AY339307
	STE-U 3938							
<i>P. diplodiopsis</i>	CBS 590.84 ³ ,	<i>V. vinifera</i>	Italy	Unknown	AY339288	AY339334	AY339359	AY339308
	STE-U 3940							
<i>P. eucalyptorum</i>	STE-U 610	<i>Eucalyptus</i>	Brazil	P. W. Crous		AY339335		
<i>P. eucalyptorum</i>	STE-U 1334	<i>Eucalyptus</i>	Indonesia	M. J. Wingfield		AY339336		

Continued

Table 1. Continued

Species	Accession no. ¹	Substrate	Country	Collector	Areas sequenced ²			
					LSU	ITS	EF1- α	H3
<i>P. eucalyptorum</i>	CBS 112640 ³ , STE-U 3904	<i>Eucalyptus</i>	Australia	P. Q. Thu & R. J. Gibbs	AY339290	AY339338		
<i>P. eucalyptorum</i>	STE-U 3905	<i>Eucalyptus</i>	Vietnam	M. J. Dudzinski & P. Q. Thu	AY339289	AY339339	AY339361	
<i>P. eucalyptorum</i>	IMI 111024, STE-U 3906	<i>Eucalyptus</i>	Australia	P. Q. Thu & R. J. Gibbs		AY339340		
<i>P. eucalyptorum</i>	STE-U 3912	<i>Eucalyptus</i>	Brazil	A. C. Alfenas		AY339341		
<i>P. granati</i>	ATCC 12685, CBS 252.38 STE-U 3714	<i>Vitis vinifera</i>	Italy	G. Goidanich	AY339291	AY339342	AY339362	
<i>P. macrospora</i>	CBS 524.73 ³ , STE-U 3935	<i>Terminalia</i> <i>ivorienensis</i>	Ivory Coast	H. A. van der Aa	AY339292	AY339343	AY339363	
<i>Pilidiella</i> sp.	PPRI 3870, CBS 111021, STE-U 3828	<i>Fragaria</i> sp.	South Africa	C. Roux		AY339346		AY339310
<i>Pilidiella</i> sp.	IMI 100482, STE-U 4200	<i>V. vinifera</i>	India	K. S. Bilgrami	AY339295	AY339347		AY339311

Continued

Table 1. Continued

Species	Accession no. ¹	Substrate	Country	Collector	Areas sequenced ²			
					LSU	ITS	EF1- α	H3
<i>Schizoparme</i> <i>straminea</i> (<i>P. castaneicola</i>)	CBS 149.22 ³ , STE-U 3932	Unknown	USA	C. L. Shear	AY339296	AY339348	AY339366	AY339312
<i>Schizoparme</i> <i>straminea</i> (<i>P. castaneicola</i>)	PPRI 3871, STE-U 3829	Debris	South Africa	C. Roux	AY339294	AY339345	AY339365	
<i>Schizoparme</i> <i>straminea</i> (<i>P. castaneicola</i>)	STE-U 405	<i>Eucalyptus</i>	South Africa	P. W. Crous	AY339293	AY339344	AY339364	AY339309

¹ ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands;
IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakenham lane, U.K.; PPRI: Plant Protection Research Institute, Pretoria, South Africa; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa;
² Sequence data of: internal transcribed spacer region (ITS), histone gene (H3), elongation factor 1- α (EF1- α); and large subunit (LSU).
³ Ex-type cultures.

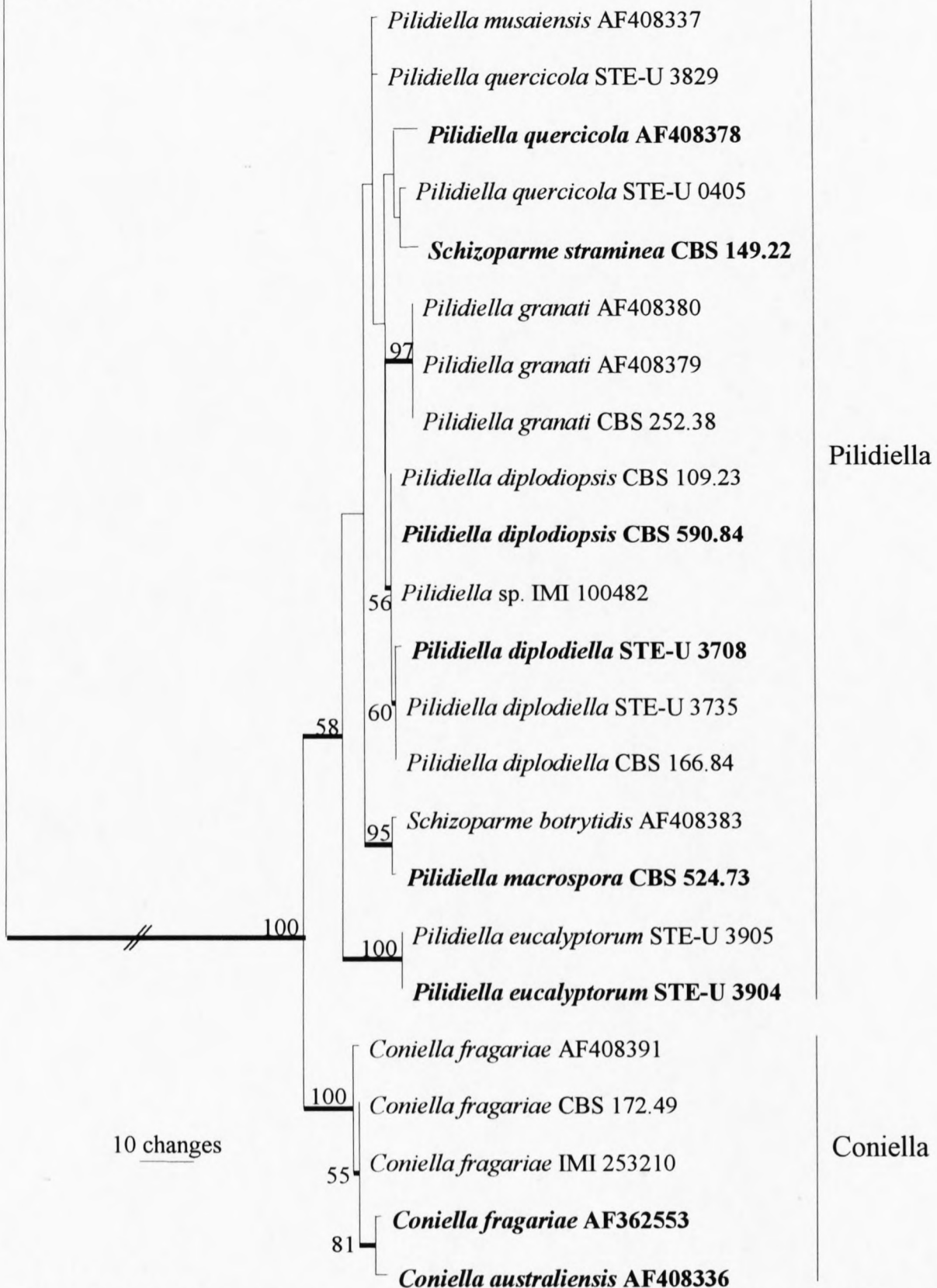
Magnaporthe grisea AB026819*Pyricularia grisea* AF362554

Figure 1. One of 47 most parsimonious trees obtained from the large subunit rRNA gene sequence data (TL = 178 steps, CI = 0.848, RI = 0.904, RC = 0.767). The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. Ex-type cultures are indicated in bold. The sequences of *Magnaporthe grisea* AB026819 and *Pyricularia grisea* AF362554 were included as outgroups.

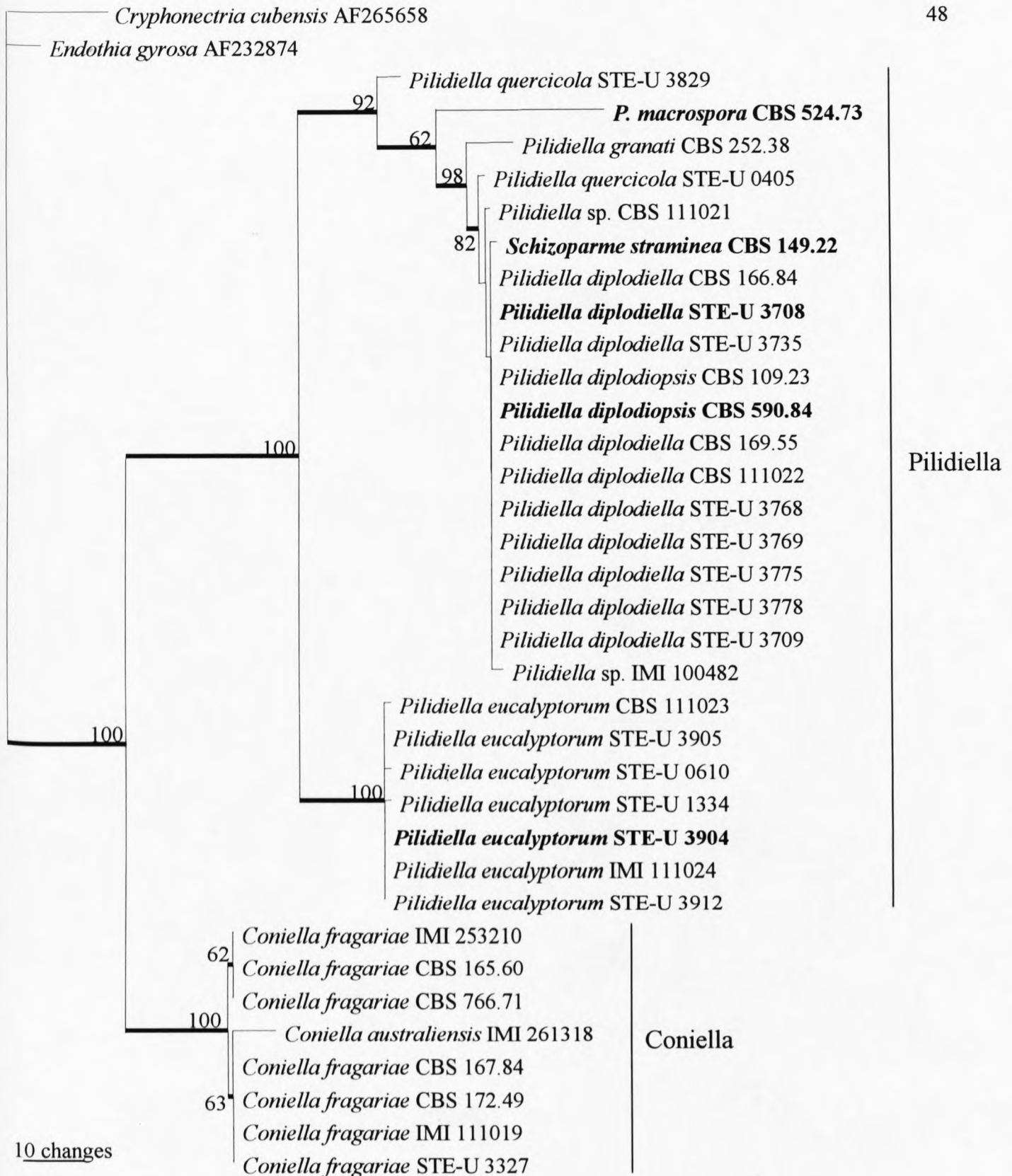


Figure 2. One of six most parsimonious trees obtained from ITS sequence data (TL = 192 steps, CI = 0.859, RI = 0.965, RC = 0.829). The numbers at the nodes represent bootstrap support values based on 1000 resamplings. Branches that appear in the strict consensus tree are indicated by thickened lines. Ex-type cultures are indicated in bold. The bar indicates 10 changes. The tree is rooted to *Cryphonectria cubensis* AF265658 and *Endothia gyrosa* AF232874.

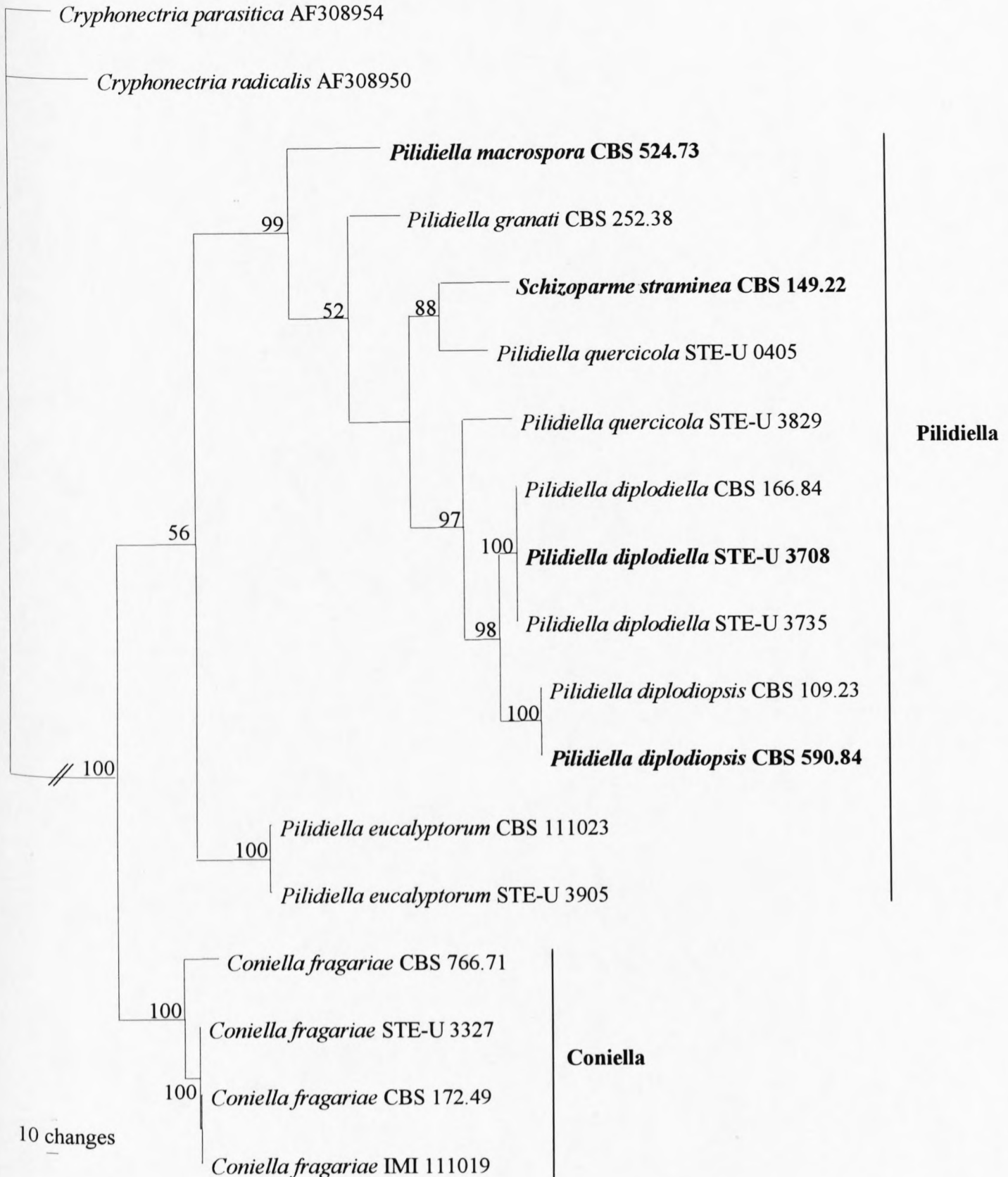


Figure 3. Single most parsimonious tree obtained from elongation factor 1- α sequence data (TL = 1056 steps, CI = 0.701, RI = 0.785, RC = 0.550). Ex-type cultures are indicated in bold. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. The tree is rooted to two *Cryphonectria* species.

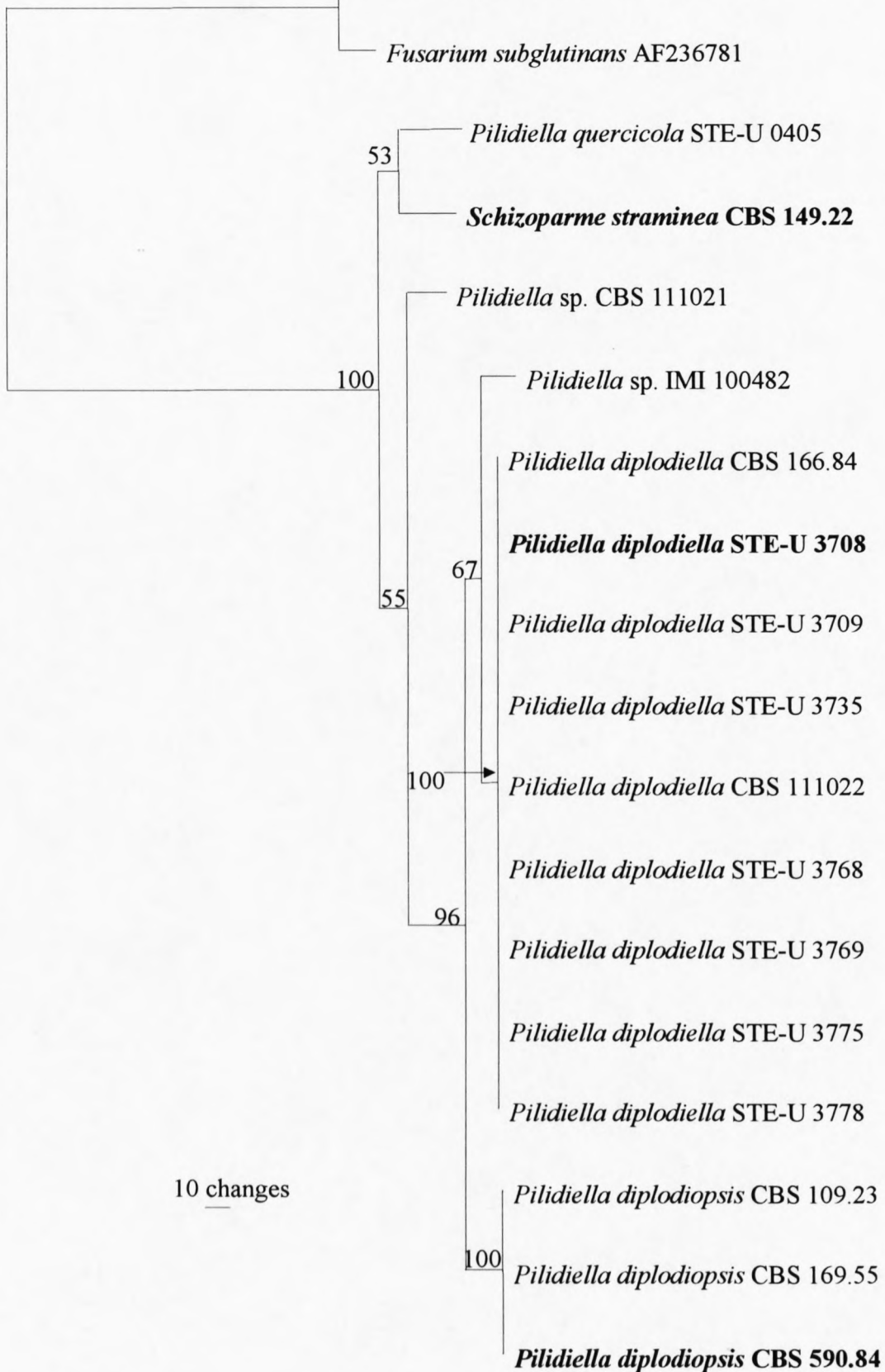
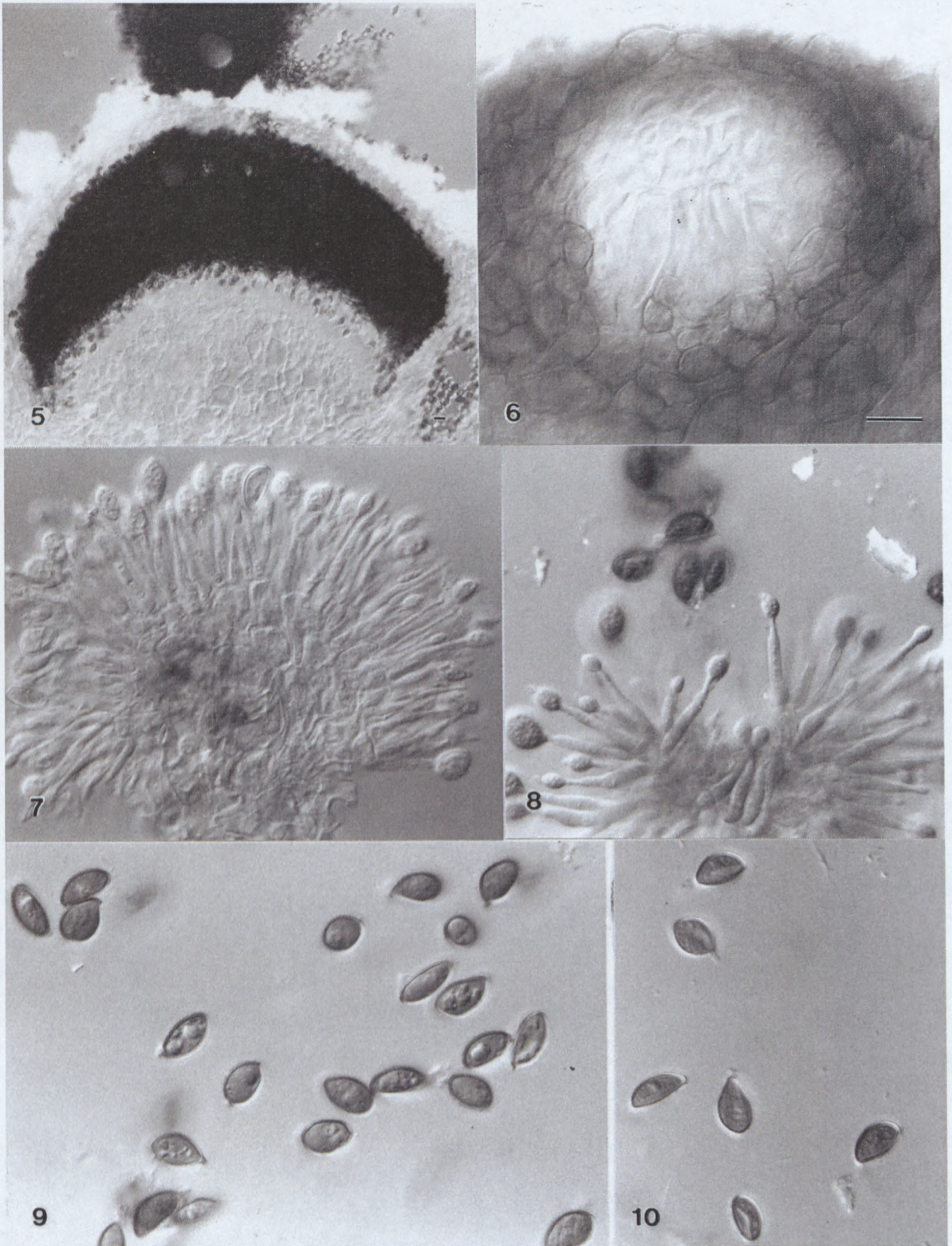


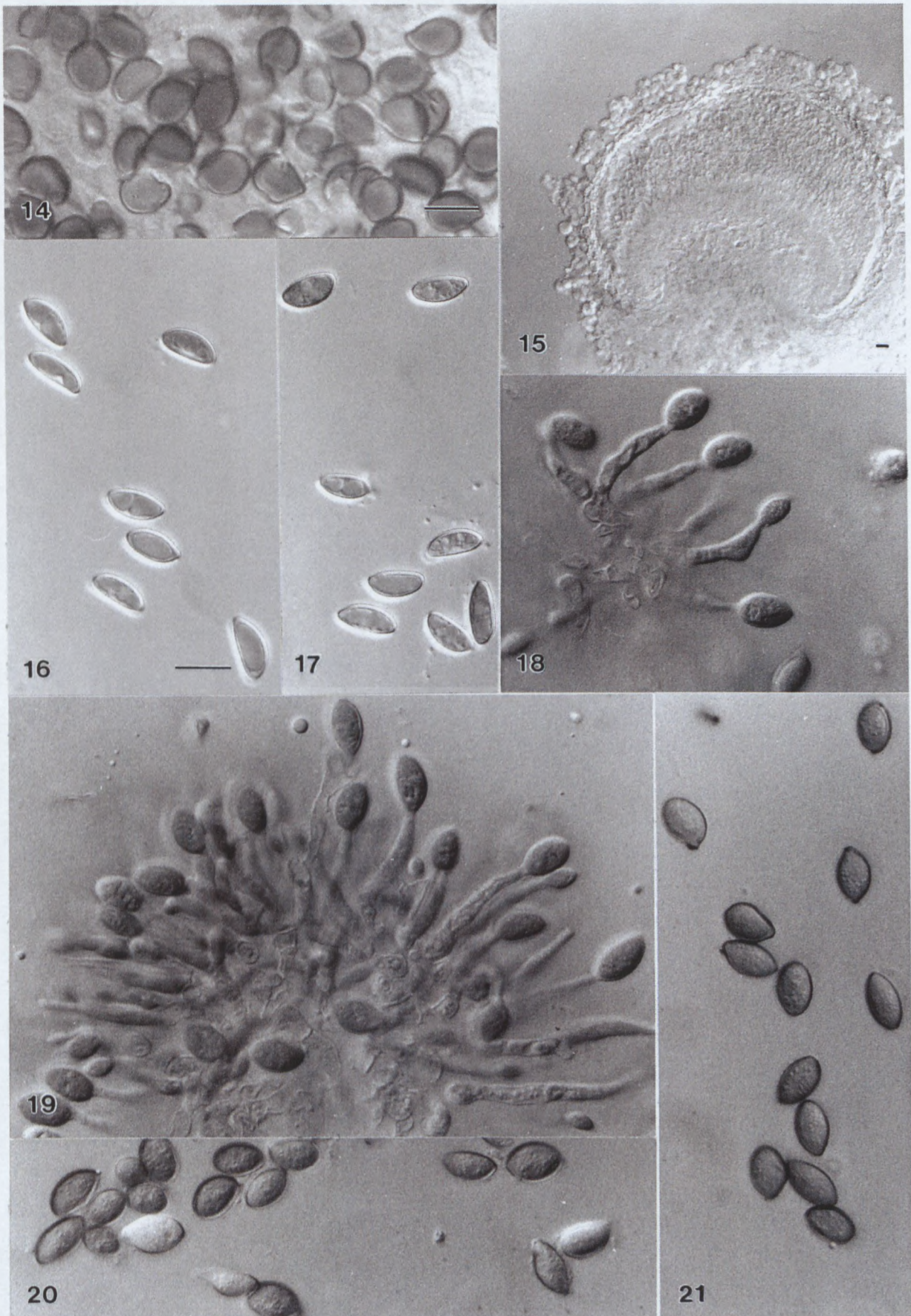
Figure 4. Single most parsimonious tree obtained from histone sequence data (TL = 462 steps, CI = 0.918, RI = 0.919, RC = 0.844). Ex-type cultures are indicated in bold. The bar indicates 10 changes. The numbers at the nodes represent bootstrap support values based on 1000 resamplings. The tree is rooted to *Fusarium proliferatum* AF291059 and *Fusarium subglutinans* AF236781.



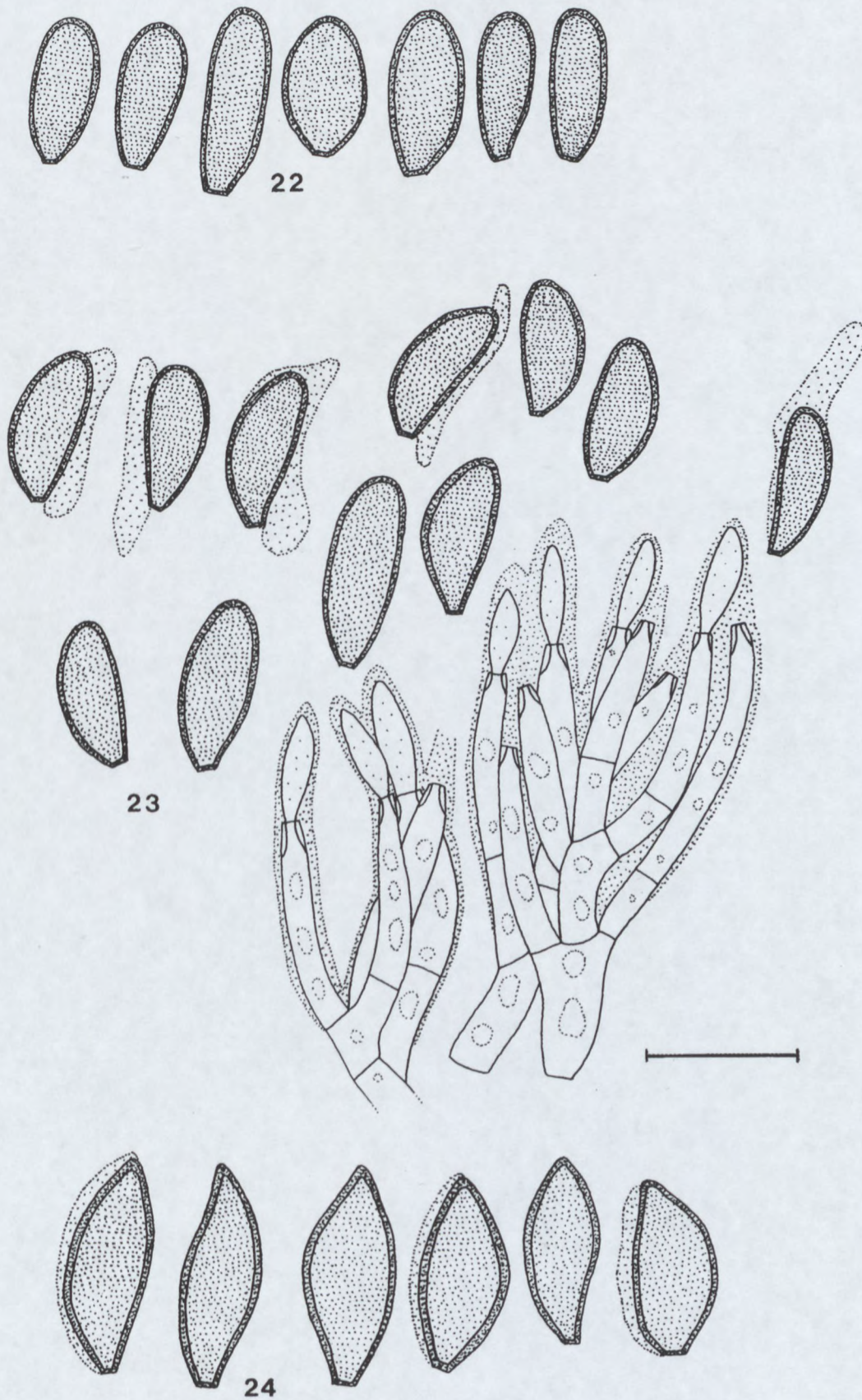
Figures 5–10. *Coniella fragaria*. **Fig. 5.** Vertical section through a pycnidium. **Fig. 6.** Ostiolar area. **Figs 7–8.** Conidiogenous cells covered in mucous. **Figs 9–10.** Conidia. Bar = 10 μm .



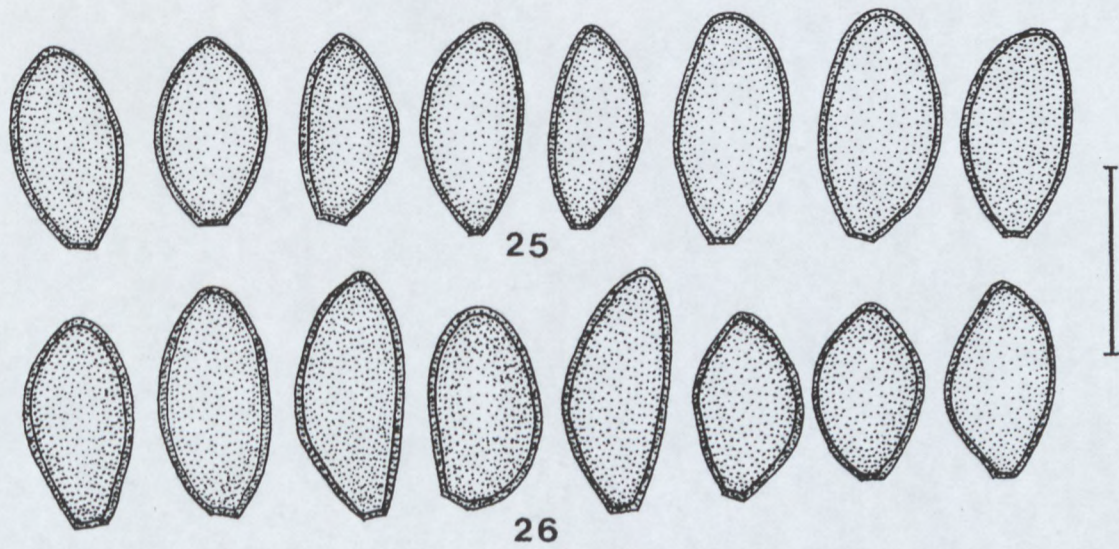
Figures 11–13. *Coniella* spp. **Fig. 11.** Conidia, conidiophores and spermatia of *C. fragariae* (STE-U 3772). **Fig. 12.** Conidia and conidiophores of *C. fragariae* (CBS 766.31). **Fig. 13.** Conidia and conidiophores of *C. australiensis* (IMI 261318). Bar = 10 μ m.



Figures 14–21. Morphological structures of *Coniella* and *Pilidiella* spp. **Fig. 14.** Conidia of *C. australiensis*. **Fig. 15.** Vertical section through a pycnidium of *P. diplodiella*. **Figs 16–17.** Conidia of *P. diplodiella*. **Figs 18–19.** Conidiogenous cells of *P. eucalyptorum* showing mucous sheaths. **Figs 20–21.** Conidia of *P. eucalyptorum*. Bar = 10 μ m.



Figures 22–24. Conidia and conidiophores of *Pilidiella* spp. **Fig. 22.** *P. diplodiella* (WINF(M) 7526, holotype). **Fig. 23.** *P. diplodiella* (epitype). **Fig. 24.** *P. petrakioidea* (PREM 47146). Bar = 10 μ m.



Figures 25–26. Conidia of *Pilidiella diplodiopsis*. **Fig. 25.** STE-U 3940 (ex-type). **Fig. 26.** STE-U 3938. Bar = 10 μm .

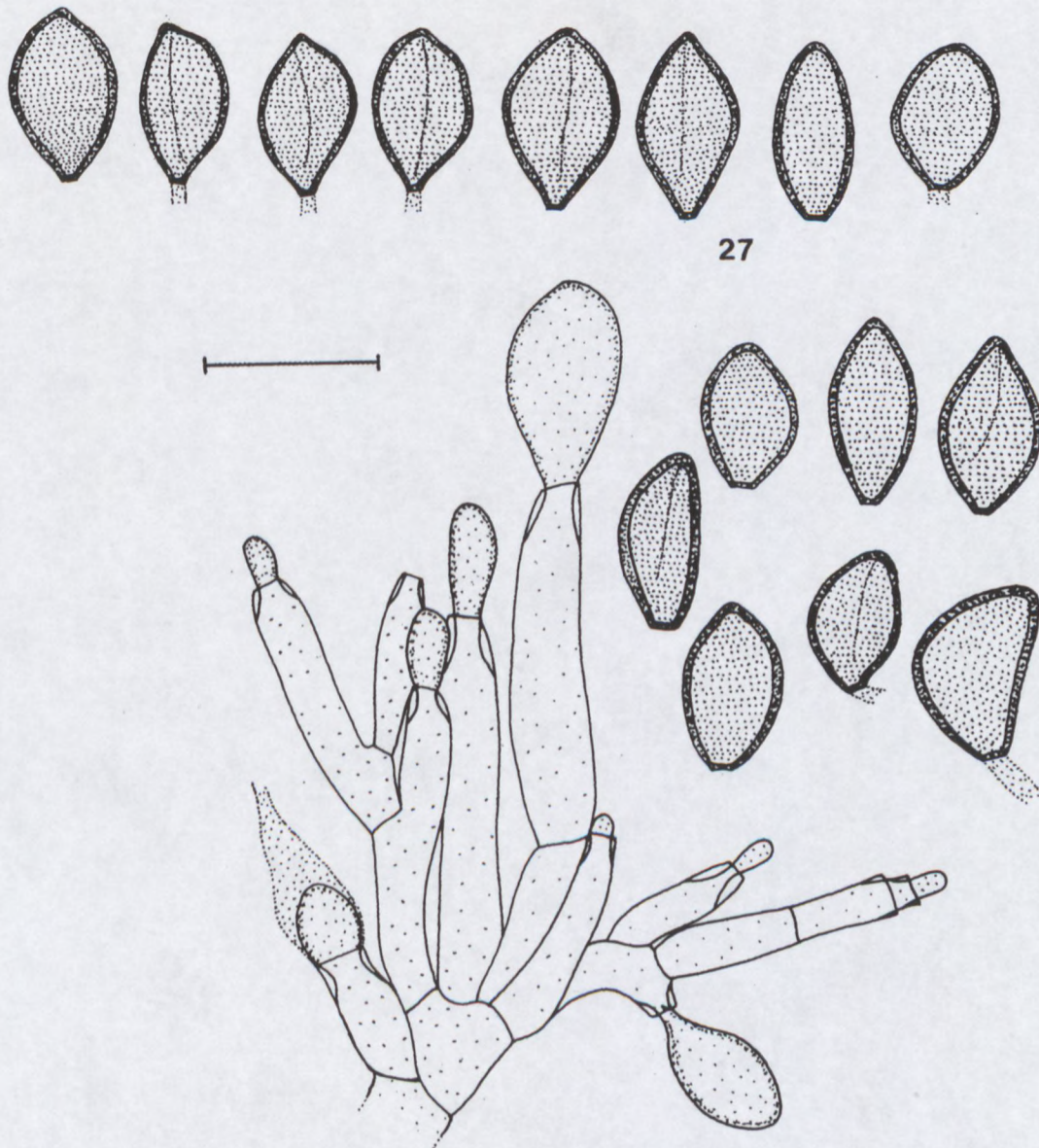
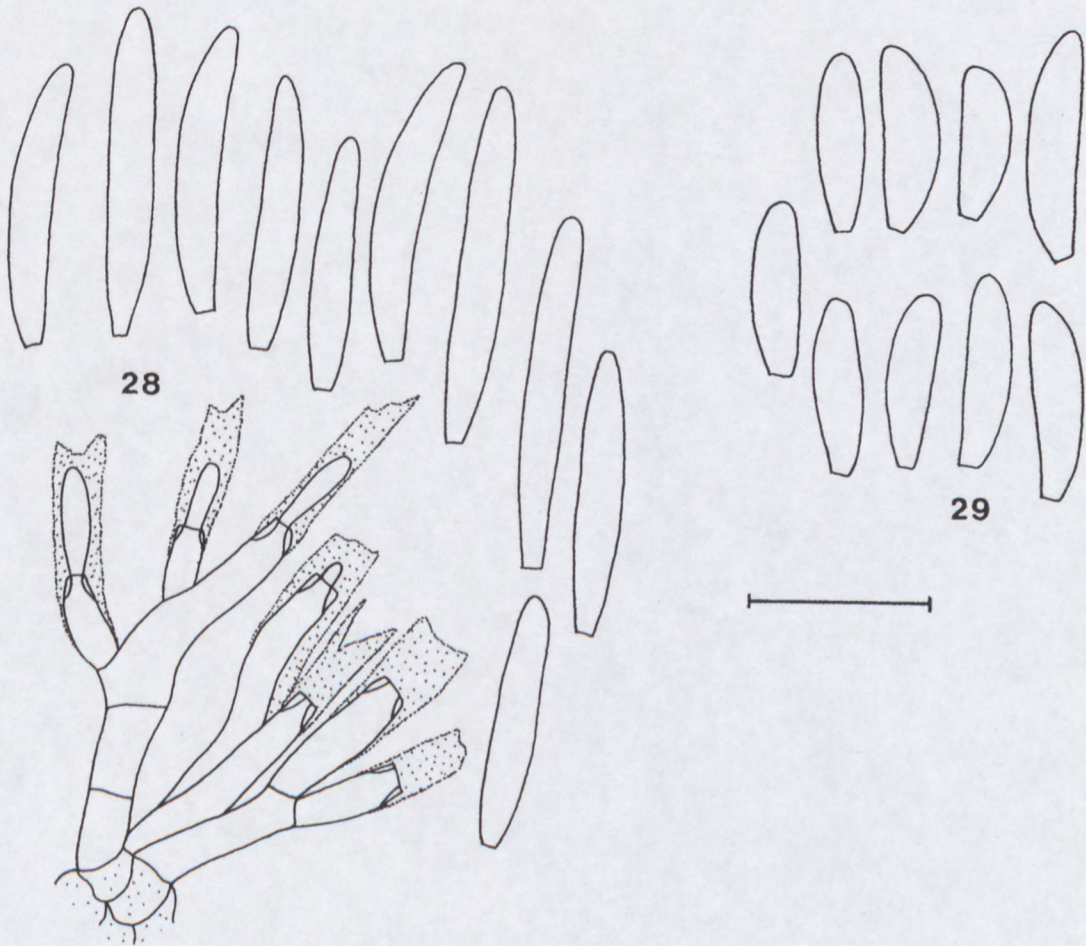
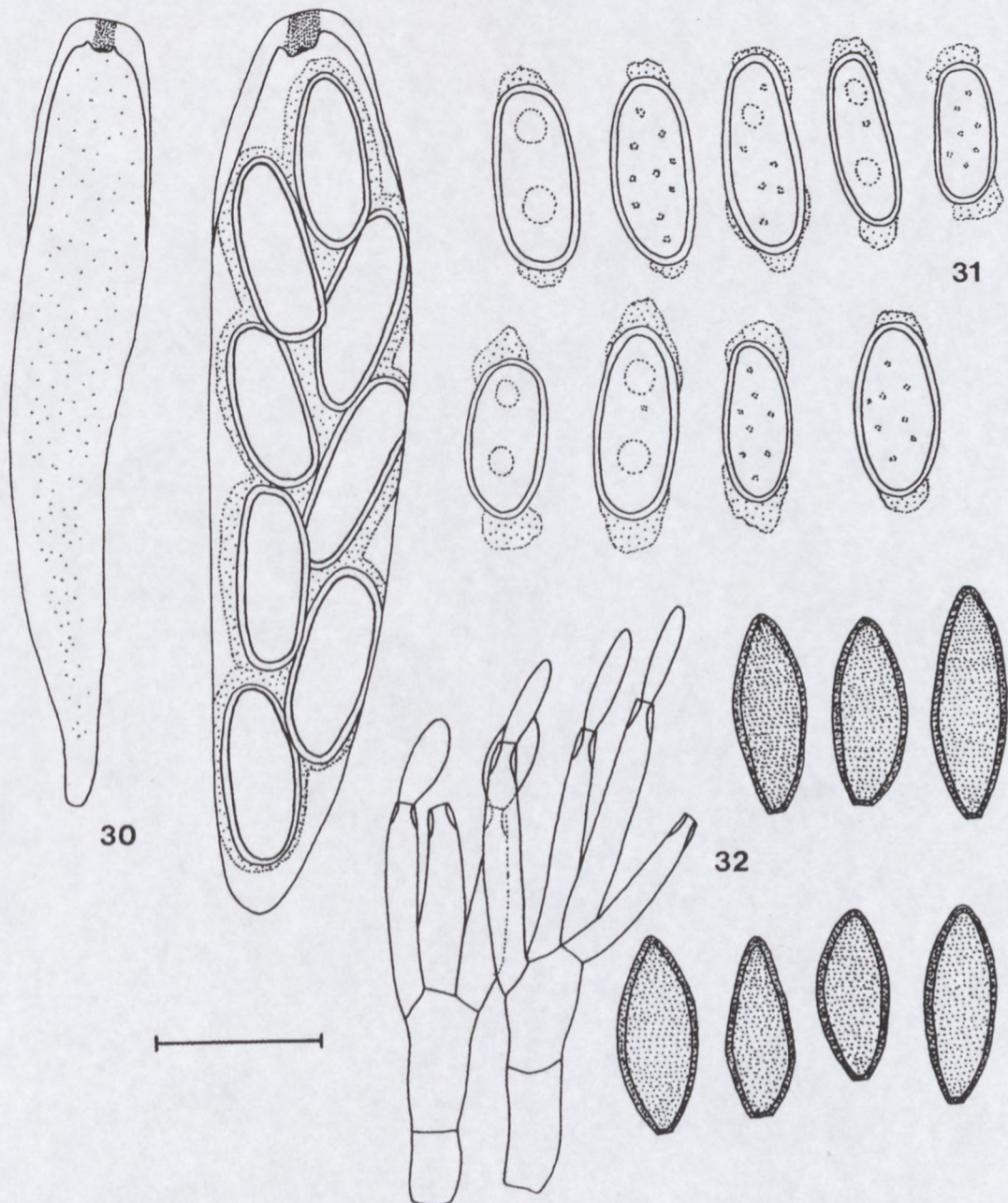


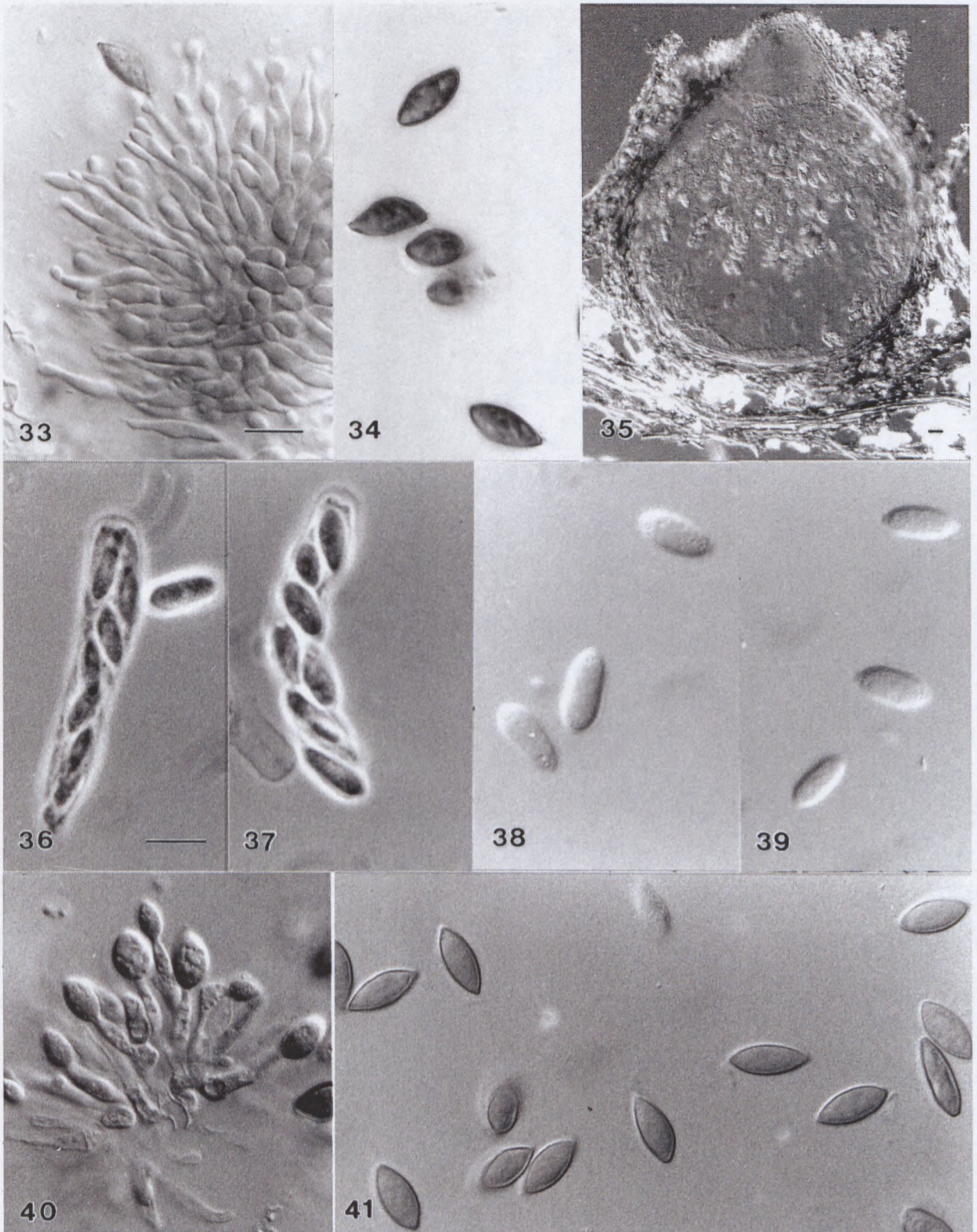
Figure 27. Conidia and conidiophores of *Pilidiella eucalyptorum*. Bar = 10 μm .



Figures 28–29. Conidia and conidiophores of *Pilidiella* spp. **Fig. 28.** *P. quercicola* (IMI 233050). **Fig. 29.** *P. granati* (CBS 252.38). Bar = 10 μ m.



Figures 30–32. *Schizoparme destruens* and its anamorph, *Pilidiella destruens*. Fig. 30. Asci. Fig. 31. Ascospores. Fig. 32. Conidia and conidiophores. Bar = 10 μ m.



Figures 33–41. Morphological structures of *Pilidiella petrakioidea* and *Schizoparme destruens*. **Fig. 33.** Conidiogenous cells of *P. petrakioidea*. **Fig. 34.** Conidia of *P. petrakioidea*. **Fig. 35.** Vertical section through a perithecium of *S. destruens*. **Figs 36–37.** Asci of *S. destruens*. **Figs 38–39.** Ascospores of *S. destruens*. **Fig. 40.** Conidiogenous cells of *C. destruens*. **Fig. 41.** Conidia of *C. destruens*. Bar = 10 μ m.

3. DNA PHYLOGENY, MORPHOLOGY AND PATHOGENICITY OF *BOTRYOSPHAERIA* SPECIES ON GRAPEVINES

ABSTRACT

Several species of *Botryosphaeria* are known to occur on grapevines and can cause a wide range of disorders, including bud mortality, dieback, brown wood streaking and bunch rot. In the present study, 11 *Botryosphaeria* spp. associated with grapevines growing in various parts of the world, but primarily in South Africa, are distinguished based on morphology, DNA sequences (ITS-1, 5.8S, ITS-2 and EF1- α) and pathological data. *Botryosphaeria australis*, *B. lutea*, *B. obtusa*, *B. parva*, *B. rhodina* and a *Diplodia* sp. are confirmed from grapevines in South Africa, while *Diplodia porosum*, *Fusicoccum viticlavatum* and *F. vitifusiforme* are described as new. Although isolates of *B. dothidea* and *B. stevensii* are confirmed from grapevines in Portugal, neither of these species occurred in South Africa, nor were any isolates of *B. ribis* confirmed from grapevines. All grapevine isolates from Portugal, formerly presumed to be *B. ribis*, are identified as *B. parva* based on their EF1- α sequence data. From artificial inoculations on grapevine shoots, I conclude that *B. australis*, *B. parva*, *B. ribis* and *B. stevensii* are more virulent than the other species studied. The *Diplodia* sp. collected from grapevine canes is morphologically similar, but phylogenetically distinct from *D. sarmentorum*. A culture of *Diplodia sarmentorum*, considered to be authentic for the species by Wollenweber, is shown to be the same as Booth's culture of *Othia spiraeae*, the type species of the genus *Othia* (Botryosphaeriaceae). Booth's strain of *O. spiraeae*, which formed *D. sarmentorum* in culture, clustered within *Botryosphaeria*, and is thus regarded as probable synonym. These findings confirm earlier suggestions that the generic concept of *Botryosphaeria* should be expanded to include genera with septate ascospores and *Diplodia* anamorphs.

INTRODUCTION

Members of the genus *Botryosphaeria* Ces. & De Not. are known to be cosmopolitan, having broad host ranges and wide geographical distributions (Barr, 1972; 1987). Symptoms caused by *Botryosphaeria* species on grapevines include bud mortality, dieback, brown streaking inside the wood, internal necrotic lesions and in some cases even bunch rot (Lehoczky, 1988;

Phillips, 1998; 2000; Castillo-Pando *et al.*, 2001), while leaf spots, cankers, dieback, and various other fruit, shoot and trunk diseases are common on other hosts (von Arx, 1987; Denman *et al.*, 2000). Several species that are considered to be saprotrophic have been reported from grapevines, while others have been shown to be severe pathogens of this host. Species of *Botryosphaeria* readily infect wounds, and in the case of grapevines, this is especially true for pruning wounds (Castillo-Pando *et al.*, 2001; Phillips, 2002). Symptoms usually develop slowly, and severe symptoms only become visible in grapevines that are eight or more years old, or that are subjected to stress (Boyer, 1995; Larignon & Dubos, 2001). Common species known from grapevines include *B. dothidea* (Moug.: Fr.) Ces. & De Not., *B. parva* Pennycook & Samuels, *B. obtusa* (Schwein.) Shoemaker, *B. stevensii* Shoemaker, *B. lutea* A.J.L. Phillips and *B. ribis* Grossenb. & Duggar (Pascoe, 1998; Phillips, 2002).

In spite of the range of symptoms associated with species of *Botryosphaeria*, field diagnosis of the causal organism is difficult, as symptoms often resemble those of other diseases such as Petri disease, caused by *Phaeomoniella chlamydospora*, Phomopsis cane and leaf spot, caused by *Phomopsis viticola* (Sacc.) Sacc., or Eutypa dieback, caused by *Eutypa lata* (Pers.) Tul. & C. Tul. (Castillo-Pando *et al.*, 2001). Accurate identification of the causal species is also difficult since *Botryosphaeria* teleomorphs are rarely encountered in nature (Shoemaker, 1964; Jacobs & Rehner, 1998) and teleomorphs rarely form in culture. The diversity among these teleomorphs is also insufficient to allow clear differentiation at species level (Shoemaker, 1964; Laundon, 1973). Thus, the taxonomy and identification of *Botryosphaeria* species is based mainly on the anamorphic characters (Denman *et al.*, 2000; Phillips, 2002), which are frequently combined with molecular data (Jacobs & Rehner, 1998; Denman *et al.*, 2003; Phillips *et al.*, 2002; Slippers *et al.*, 2004a; b). The diversity of anamorph states of *Botryosphaeria* has added to the taxonomic confusion. Seven anamorph genera have been applied to asexual states of species of *Botryosphaeria*. Recent research suggests that anamorphs of *Botryosphaeria* belong to either *Fusicoccum* Corda (hyaline, thin-walled conidia), or *Diplodia* Fr. (pigmented, thick-walled conidia) (Pennycook & Samuels, 1985; Crous & Palm, 1999; Denman *et al.*, 2000; Zhou & Stanosz, 2001; Phillips, 2002).

A major problem facing the grapevine industry remains the correct identification of the *Botryosphaeria* species causing disease on plants from different cultivars, localities and countries. Species occurring on grapevines in different countries have been shown to differ in pathogenicity, and this has led to confusion and conflicting reports about which species of *Botryosphaeria* are important pathogens of grapevines (Phillips, 2002). These species differ in their epidemiology, the disease symptoms they cause, their relative importance and the control

strategies that should be followed to combat the various diseases.

In South Africa, several species of *Botryosphaeria* have been reported as pathogens of grapevines, including *B. obtusa*, *B. dothidea* and *B. ribis* (Crous *et al.*, 2000), as well as *B. vitis* (Schulzer) Sacc. (Doidge, 1950). *Botryosphaeria* is regarded as an important pathogen of grapevines in South Africa and is frequently isolated from grapevines with canker and dieback symptoms (Fourie & Halleen, 2001). The identity of the various causal species, however, as well as their relative importance, remains unknown. The aims of this study were to use molecular methods and morphological characteristics to compare South African *Botryosphaeria* isolates with those associated with grapevine diseases elsewhere and to determine which species should be regarded as potentially important pathogens of this host.

MATERIALS AND METHODS

Isolates

Isolations were made routinely for the past 5 years from symptomatic material of diseased grapevines (Table 1). Some isolates were also obtained from young asymptomatic nursery plants (shoots). Plant tissue was surface-sterilised by placing in 70% ethanol for 30 s, 1% NaOCl for 1 min and again in 70% ethanol for 30 s before drying under a laminar flow hood. Small pieces of tissue were taken from the margin between necrotic and apparently healthy tissue and plated onto 2% potato dextrose agar (PDA; Biolab, Midrand, South Africa). Hyphae growing out from the tissue pieces were subcultured onto fresh PDA plates, incubated, and hyphal-tipped in order to obtain pure cultures. In order to enhance sporulation, isolates were plated out on water agar (WA; Biolab) plates, to which 3 cm pieces of double-autoclaved pine needles were added. The plates were incubated at 25°C under near-ultraviolet light in a 12 hr light–darkness regime for 2–3 wks. The 95% confidence intervals of conidial dimensions were derived from at least 30 observations at 1000 × magnification. Growth rates, cultural characteristics and cardinal temperatures for growth were determined for isolates plated onto PDA in 90 mm diameter Petri dishes and incubated in the dark for 7 days at seven different temperatures, ranging from 5°C to 35°C in 5°C intervals. Three plates were used for each isolate at each temperature. Radial mycelial growth was measured perpendicularly for each plate, and the mean calculated to determine the growth rates for each species. The experiment was repeated once. Colony colors were described from isolates incubated at 25°C under near-ultraviolet light for 7 days, according to Rayner (1970). Cultures are

maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U) and at the Centraalbureau voor Schimmelcultures in Utrecht, the Netherlands (CBS) (Table 1).

Sequence comparisons

A total of 122 *Botryosphaeria* isolates were used for ITS sequence analysis (Table 1), their phylogeny determined (results not given), and a subset of 39 chosen for analysis of the translation elongation factor 1- α (EF1- α) gene. The isolation protocol of Lee and Taylor (1990) was used to extract genomic DNA from fungal mycelia grown on PDA. The primers ITS1 and ITS4 were used to amplify part of the nuclear rRNA operon using the PCR conditions recommended by the authors (White *et al.*, 1990). The primers EF1-728F and EF1-986R (Carbone & Kohn, 1999) were used to amplify part of the EF1- α gene. PCR conditions were the same for this region, except for the MgCl₂ concentration, which was increased to 4.0 mM. PCR products were separated by electrophoresis at 80 V for 1 h in a 0.8% (w/v) agarose gel in 0.5 \times TAE running buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, United Kingdom) following ethidium bromide staining.

The amplification products were purified using a NucleoSpin® Extract 2 in 1 kit (Macherey-Nagel, Germany). The purified products were sequenced in both directions using the PCR primers and the cycle sequencing reaction was carried out as recommended by the manufacturer with an ABI Prism Big Dye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, California) containing AmpliTaq DNA Polymerase. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut).

The ITS and EF1- α sequences were assembled and added to the outgroup sequences, *Cercospora beticola* Sacc. (STE-U 5073) and *Cercospora penzigii* Sacc. (STE-U 4001), using Sequence Alignment Editor v2.0a11 (Rambaut, 2002) and manual adjustments for improvement were made where necessary. The phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all datasets using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping

algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis & Bull, 1993). Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) also were calculated. The resulting trees were printed with TreeView Version 1.6.6 (Page, 1996). A partition homogeneity test was conducted in PAUP (Swofford, 2000) to examine the possibility of a joint analysis of the ITS and EF1- α data sets.

Pathogenicity

In vitro screening on green shoots. A total of 21 isolates, representing 8 different species of *Botryosphaeria* (Tables 2, 3), were selected for pathogenicity screening. Isolates were plated on PDA and incubated at 25 C for 1 week. Inoculations were made on green shoots of the grapevine cultivar 'Periquita'. Shoots were cut from vines *ca* 2 months after budburst and sections (internodes 4–6) were used for inoculations. A total of 12 shoot sections were used for each species. Shoot sections were wounded on internode five (2 mm deep) with a 4 mm cork-borer. A colonised agar plug, cut from a 1-week-old culture was placed in the wound and covered with Parafilm. Inoculated shoots were incubated in the dark under moist conditions in the laboratory for 10 days at 23°C. Following the incubation period, the shoot sections were split longitudinally through the wound and the internal lesions measured. The layout of the trial was a completely randomised design and the data were statistically analysed using SAS (SAS, 1999). An analysis of variance and Student's t-tests for least significant differences were done. Following the experiment, all plant material was destroyed by autoclaving it twice for 30 min.

In vitro screening on mature canes. A set of 16 *Botryosphaeria* isolates were selected to use in this experiment (Tables 4, 5). Three isolates were, however, selected for *B. australis* and *B. parva*, as these species were most commonly isolated from diseased vines. Inoculations were done using mature canes of 2 grapevine cultivars, 'Cabernet Sauvignon' and 'Chardonnay'. Four canes were inoculated per cultivar for each isolate. Canes used for inoculations were cut from vines *ca* 2 months after harvest. Canes were wounded on internode five (2 mm deep) with a 4 mm cork-borer and inoculated as described above. Inoculated canes were incubated in the dark under moist conditions in the laboratory for 3 weeks under strict quarantine conditions. Following this period, the canes were assessed and the data analysed as described above.

In vivo pathogenicity on mature vines. For this trial the same set of 21 isolates was used as in the in vitro green shoots trial (Table 3). The trial layout and number of replicates per species

were also the same. Inoculations were made in a vineyard on 15-year-old grapevine plants of the cultivar 'Periquita'. Inoculations were made on mature canes in the same manner as with the green shoots trial, but the canes were left attached to the plant. Inoculations were also made in mature wood by drilling a hole 4 mm wide and 1.5 cm deep into the arms of the vines. A colonised agar plug, cut from a 1-week-old culture, was placed in the wound. The wound was sealed with petroleum jelly and covered with Parafilm. After a period of 6 months, the inoculated arms and mature canes were collected. The canes were assessed for disease severity and the data analysed as described above. Re-isolations were made from the leading edges of lesions and the cultures identified by inducing sporulation in the same manner as for the morphological descriptions, in order to satisfy Koch's postulates. All plant material was destroyed by autoclaving it twice for 30 min.

RESULTS

Phylogenetic analysis

Approximately 550 and 300 bases were determined for the ITS region and EF 1- α gene, respectively, of the isolates (Table 1). The manually adjusted alignment contained 41 isolates with 529 characters for the ITS region, and 357 characters for EF1- α , including alignment gaps (data not shown). New ITS and EF1- α sequences were deposited in GenBank (Table 1) and the alignments in TreeBASE (SN 1533). The result of the partition homogeneity test ($P = 0.22$, where $P \geq 0.05$) was significantly incongruent, indicating that the ITS and EF1- α data sets could be combined.

The combined data set contained 886 characters, of which 498 were parsimony-informative, 11 were variable and parsimony-uninformative and 377 were constant. Maximum parsimony analysis of the combined sequence data resulted in a single most parsimonious tree (Fig. 1). The first clade (100% bootstrap support) contained two *B. rhodina* isolates (STE-U 5051, 4419). The second clade (100% bootstrap support) contained a *B. stevensii* isolate (STE-U 5038) isolated from grapevines in Portugal, which grouped separately from two isolates of *B. obtusa* (STE-U 5034, 4542), which formed a well-supported clade (100% bootstrap support). Two isolates of *Diplodia porosum* (STE-U 5046, 5132) isolated from grapevine pruning debris, again grouped separately (100% bootstrap support). The next clade (STE-U 5148, 5048) contained 2 isolates of a *Diplodia* sp. (100% bootstrap support), which was also obtained from pruning debris,

and clustered close to (100% bootstrap support) *Othia spiraeae* (Fuckel) Fuckel (IMI 63 581) and its anamorph, *Diplodia sarmentorum* (Fr.) Fr. (CBS 120.41) (100% bootstrap support). *Botryosphaeria dothidea* was represented by one grapevine isolate from Portugal (STE-U 4595), and another from Argentina (STE-U 5045). However, no isolates of *B. dothidea* were isolated from vines in South Africa.

The two new *Fusicoccum* species isolated from grapevines, namely *F. viticlavatum* (STE-U 5041, 5044) and *F. vitifusiforme* (STE-U 5252, 5050), each clustered with 100% bootstrap support. Isolates of *B. lutea*, including the ex-type strain (STE-U 4593), also clustered with 100% bootstrap support, adjacent to *B. australis* Slippers, Crous & M.J. Wingf. (72% bootstrap support) (Slippers *et al.*, 2004b).

Isolates of *B. ribis* and *B. parva* could be separated based on their EF1- α data. The *B. ribis* clade was supported by a bootstrap value of 100% and the *B. parva* clade with 62%. The South African isolates from grapevines all fell into the *B. parva* clade. No isolates of *B. ribis* were isolated from grapevines in South Africa.

Taxonomy

***Botryosphaeria vitis* (Schulzer) Sacc., Syll. Fung. 1:463. 1882**, homonym of *B. vitis* Niessl, in Beitr.:48. 1871.

Basionym: *Gibbera vitis* Schulzer, in Verh. Zool.-Bot. Ges Wien 20:642. 1872.

Specimens examined. SOUTH AFRICA. WESTERN CAPE: Stellenbosch, Banhoek, on canes of *Vitis vinifera* L., *Buller*, PREM 46 581.

Notes. *Botryosphaeria vitis* Niessl has 1-septate, oblong ascospores, $14\text{--}16 \times 4\text{--}5 \mu\text{m}$, and subsequently was placed in *Lisea* Sacc. (= *Nectria fide* Rossman *et al.*, 1999) as *L. vitis* (Niessl) Sacc. (*Michelia* 1:43. 1879). The name proposed by Saccardo as *B. vitis* (Schulzer) Sacc. (1882) is thus illegitimate, and cannot be used. Nevertheless, Doidge (1950) reported *B. vitis* (Schulzer) Sacc. as occurring on grapevines in the Stellenbosch region of South Africa. Saccardo (1882) cited this species as having ovate ascospores, $26\text{--}27 \times 10\text{--}13 \mu\text{m}$. Upon examination of the South African material (PREM 46 581), ascospores were found to be $16\text{--}25 \times 6\text{--}10 \mu\text{m}$, thus significantly smaller than those originally reported for *B. vitis*. Furthermore, conidia were hyaline, fusoid-ellipsoidal, $(17\text{--})19\text{--}22\text{--}(23) \times (5\text{--})6\text{--}6.5\text{--}(7) \mu\text{m}$ (l:w ratio 3.3), thus closely resembling the *B. lutea* – *B. australis* Slippers, Crous & M.J. Wingf. complex, both of which are shown to occur on vines. Slippers *et al* (2004b) distinguished these two species by

B. australis (l: w = 4.8) having conidia with a higher length : width ratio than *B. lutea* (l:w = 3.3), which suggests that the South African grapevine specimen is best accommodated in *B. lutea*.

***Diplodia porosum* J.M. van Niekerk & Crous, sp. nov.**

Figs. 2–8

Pycnidia solitaria, globosa vel obpyriformia, ad 400 µm diam. Cellulae conidiogenae holoblasticae, hyalinae, subcylindricae vel ampulliformes, 6–10 × 5–7 µm. Conidia hyalina, guttulata, ovoidea vel late ellipsoidea, sursum hebetate rotundata, ad basim raro truncata; pariete 2 µm crasso, multis poris 1 µm latis perforata, deinde brunnescentia, (38–)42–45(–47) × (20–)22–25(–30) µm in vitro (long: latitudo = 1.9).

Pycnidia solitary, unilocular, ostiolate, globose to obpyriform, up to 400 µm wide; pycnidial wall 4–8 cell layers thick, of dark brown textura angularis, becoming hyaline towards inner region. Conidiophores reduced to conidiogenous cells. Conidiogenous cells lining cavity, holoblastic, hyaline, subcylindrical to ampulliform, 6–10 × 5–7 µm, rarely proliferating percurrently. Conidia hyaline, guttulate, ovoid to broadly ellipsoid with a bluntly rounded apex, and flattened base; wall 2 µm thick, with pores 1 µm wide; becoming medium brown with age, (38–)42–45(–47) × (20–)22–25(–30) µm in vitro (l: w = 1.9). Colonies flat with undulating margins, dark green (27'' i) on the surface and dull green (27'' m) underneath, reaching a radius of 32 mm after 3 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 30°C, opt. 25°C

Holotype. SOUTH AFRICA. WESTERN CAPE PROVINCE: Stellenbosch, on *Vitis vinifera*, 2002, J.M. Van Niekerk, herb. CBS 7754, culture ex-type STE-U 5132, CBS 110 496.

Hosts. *Vitis vinifera*.

Known distribution. South Africa (Western Cape Province).

Notes. This species is unique within the genus *Diplodia* because of its large, thick-walled conidia with large pores (1 µm wide) and are clearly visible by light microscopy (Figs. 7, 8). Conidia are initially hyaline, but become pigmented while still in the pycnidial locule.

***Diplodia* sp.**

Figs. 9–10

Hosts. *Vitis vinifera*.

Known distribution. South Africa (Western Cape Province).

Notes. Isolates of this *Diplodia* sp. had ovoid, brown, 1-septate conidia that are 18–22 \times 10–12 μm , thus closely matching the description of *D. sarmentorum* (Wollenweber, 1941; Booth, 1958). In culture colonies are flat with undulating margins, dull green (27''m) on the surface, and greenish black (33''''k) underneath, reaching a radius of 33 mm after 7 d at 25°C. Cardinal temperatures for growth: min. 10°C, max. 30°C, opt. 25°C.

Diplodia sarmentorum was reported as the teleomorph of *Oothia spiraeae*, a cosmopolitan fungus with a wide host range (Booth 1958). Although morphologically similar, the grapevine *Diplodia* isolates proved to be phylogenetically distinct from *D. sarmentorum* (Fig. 1). As there are probably several cryptic species within *D. sarmentorum*, the grapevine isolates will therefore have to be compared to all of the 145 synonyms of *D. sarmentorum* (Wollenweber 1941), before their status can be resolved.

***Fusicoccum viticlavatum* J.M. van Niekerk & Crous, sp. nov.**

Figs. 11–15

Fusicocco luteo simile, sed conidiis ellipsoideis vel clavatis, in vitro (15–)16–18(–20) \times (6–)6.5–7.5(–8) μm (long.: lat. = 2.4) differens; coloniae pigmento luteo carentes.

Pycnidia embedded in host tissue, solitary, stromatic, globose, up to 450 μm wide; pycnidial wall 4–8 cell layers thick, of brown textura angularis, becoming hyaline towards inner region. Conidiophores 0–1-septate, hyaline, subcylindrical, 10–20 \times 2.5–3.5 μm . Conidiogenous cells holoblastic, hyaline, subcylindrical, 7–15 \times 2.5–3.5 μm , proliferating percurrently with 1–3 proliferations, or proliferating at same level (phialidic) with minute periclinal thickening. Conidia hyaline, guttulate, ellipsoid to clavate, widest in upper third, with an obtuse apex and flattened, subtruncate base, (15–)16–18(–20) \times (6–)6.5–7.5(–8) μm in vitro (l: w = 2.4). Colonies umbonate with undulating margins, olivaceous (21''k) on the surface, and dull green (27''m) underneath, reaching a radius of 26 mm after 3 days at 25°C. Cardinal temperatures for growth: min. 10°C, max. 35°C, opt. 30°C.

Holotype. SOUTH AFRICA. WESTERN CAPE PROVINCE: Stellenbosch, on *V. vinifera*, 2002, *F. Halleen*, herb. CBS 7755, culture ex-type STE-U 5044, CBS 112 878.

Hosts. *Vitis vinifera*.

Known distribution. South Africa (Western Cape Province).

Notes. *Fusicoccum viticlavatum* is closely related to *F. australe* Slippers, Crous & M.J. Wingf. and *F. luteum* Pennycook & Samuels (Fig. 1), but can readily be distinguished from these taxa based on its characteristic conidial shape. Conidia are ellipsoid to clavate in *F.*

viticlavatum, as opposed to the fusiform conidia in *F. luteum* and *F. australe*. Colonies of *F. viticlavatum* also do not produce any yellow pigment in culture as observed in *F. luteum* and *F. australe* (Slippers *et al.*, 2004b).

***Fusicoccum vitifusiforme* J.M. van Niekerk & Crous, sp. nov.**

Figs. 16–24

Fusicocco luteo simile, sed conidiis brevioribus, *in vitro*, (18–)19–21(–22) × (4.5–)5.5–6.5(–8) µm (long.:lat. 3.3) differens; coloniae pigmento luteo carentes.

Pycnidia solitary, stromatic, globose to obpyriform, up to 450 µm diam; pycnidial wall 6–15 cell layers thick, of brown textura angularis, becoming hyaline towards inner region. Conidiophores 0–1-septate, hyaline, subcylindrical, 10–45 × 2.5–5 µm. Conidiogenous cells holoblastic, hyaline, subcylindrical, 10–30 × 2.5–3.5 µm, proliferating percurrently with numerous proliferations, or proliferating at the same level (phialidic) with minute periclinal thickening. Conidia hyaline, granular, fusoid to ellipsoid, widest in the upper third with an obtuse apex and flattened, subtruncate base, (18–)19–21(–22) × (4.5–)5.5–6.5(–8) µm *in vitro* (l:w = 3.3). Colonies effuse with even, smooth margins, white on the surface, and greenish olivaceous (23''') underneath, reaching a radius of 31 mm after 3 days at 25°C. Cardinal temperatures for growth: min. 10°C, max. 35°C, opt. 30°C.

Holotype. SOUTH AFRICA. WESTERN CAPE PROVINCE: Stellenbosch, on *V. vinifera*, 2002, J.M. Van Niekerk, herb. CBS 7756, culture ex-type STE-U 5252, CBS 110 887.

Hosts. *Vitis vinifera*.

Known distribution. South Africa (Western Cape Province).

Notes. *Fusicoccum vitifusiforme* is closely related to *F. australe* and *F. luteum*, and also has fusiform conidia as in the case of the latter two species (Slippers *et al.*, 2004b). It is distinct, however, by not producing any yellow pigment in culture and by having conidia that are shorter (up to 22 µm in length) than those of *F. australe* (18–30 µm) and *F. luteum* (15–30 µm).

Pathogenicity

In vitro screening on green shoots. Mean lesion lengths caused by isolates of the *Botryosphaeria* spp. on green 'Periquita' shoots are given in Table 2. *Botryosphaeria australis* and *B. parva* caused significantly longer lesions (65.29 and 57.30 mm, respectively) than the other

species tested (4.30 to 17.22 mm). All of the species tested caused markedly longer lesions compared to the agar plug-treated control (3.38 mm), although not all were significantly different.

In vitro screening on mature canes. Analysis of variance showed no significant interaction between cultivar and treatment ($P = 0.9431$), and the lesion length measurements for both cultivars were therefore pooled (Table 3). The most severe lesions were caused by *B. ribis* (26.75 mm long), followed by *B. australis* (18.17 mm), *B. stevensii* (17.56 mm), and *B. parva* (11.25 mm). *Fusicoccum vitifusiforme*, *Diplodia* sp., *B. dothidea*, *B. obtusa* and *F. viticlavatum* (7.69 mm to 7.10 mm) caused smaller but still significantly longer lesions than the controls (3.81 mm).

In vivo pathogenicity on mature vines. Mean lesion lengths caused by *in vivo* inoculations with *Botryosphaeria* spp. on mature canes and mature wood of the grapevine cultivar 'Periquita' are given in Table 4. The species that caused the most severe lesions on mature canes were *B. parva* (15.01 mm long), *D. porosum*. (13.22 mm), *B. australis* (12.89 mm) and *B. rhodina* (11.73 mm). *Botryosphaeria obtusa*, the *Fusicoccum* spp. and *Diplodia* sp. caused significantly smaller lesions (8.08 mm to 7.18 mm), but still significantly larger than the agar plug-treated control (4.18 mm).

Two symptom types could be distinguished in the mature wood. The first type was black vascular streaking that originated from the inoculation site. The second symptom type was a brown wood-rotting lesion. Vascular streaking mostly extended beyond the rotting lesion. Since multiple isolates were not tested for each species, species and isolate interaction could not be determined in the analyses of variance of the rotting and streaking lesion lengths in the mature wood (Table 4). However, significant differences in the lesion lengths caused by different isolates from the same species were observed (data not shown). Mean rotting and streaking lengths are given in Table 4. *Botryosphaeria australis* caused the most severe rotting lesion (25.90 mm long), while the other species caused marginally to significantly longer lesions (6.80 mm to 13.54 mm) than the control (6.72 mm). *Botryosphaeria australis* also caused the most severe streaking (48.85 mm), with the other species causing vascular streaking of significantly larger proportions (22.06 mm to 36.60 mm) than the control (6.72 mm). Re-isolations from the mature wood were successful and all species were identified as being the same as originally used in inoculations, thereby satisfying Koch's postulates.

DISCUSSION

The present study represents the first attempt to characterise species of *Botryosphaeria* on grapevines using an extensive collection of isolates, and integrating morphology, pathology and molecular datasets. Eleven species were identified as occurring on grapevines, of which three are newly described. Because the present collection of strains had a strong bias towards South African vineyards, it is tempting to speculate that if vineyards from other countries also were sampled more intensively, it would lead to the identification of yet more species from this host. Crous *et al.* (2000) reported *B. ribis*, *B. obtusa* and *B. dothidea* as pathogens of grapevines in South Africa, of which only *B. obtusa* could be confirmed. New records from grapevines in South Africa include *B. rhodina*, *B. lutea*, *B. parva*, *B. australis*, *Diplodia* sp. (resembling *D. sarmentorum*), *D. porosum*, *F. viticlavatum* and *F. vitifusiforme*. Surprisingly, none of the collected isolates were representative of *B. dothidea* or *B. ribis*, which are the names commonly used for isolates causing Botryosphaeria dieback of grapevines (Pascoe, 1998; Phillips, 2002).

The *in vitro* and *in vivo* pathogenicity trials in this study tested the ability of the mycelium of *Botryosphaeria* spp. to infect wounded grapevine tissue at different stages of phenological development (green shoots, mature canes and mature wood). All species were successfully re-isolated from the respective lesions and should thus be considered as potential pathogens of grapevines. *Botryosphaeria australis* and *B. parva* were consistently amongst the species causing the most severe lesions on green shoots, mature canes and mature wood. However, all species showed variable degrees of lesion formation. For example, *B. rhodina* and *D. porosum* grouped amongst the most virulent species in the *in vivo* mature cane trial, but in the *in vitro* mature cane trial they grouped with the least virulent species. This variability among species might be attributed to their reaction on host tissue at different stages of phenological development, physiological character of the host tissue, cultivar susceptibility and/or conditions and length of incubation. Variability was observed in the virulence of different isolates within a species. The *B. obtusa* isolate STE-U 5139 consistently caused lesions that were twice as large as the lesions caused by the other *B. obtusa* isolates (STE-U 4444 and STE-U 4440, results not shown). This phenomenon was also observed for *B. australis*, where isolate STE-U 5040 caused lesions twice as large as the other *B. australis* isolates (STE-U 4598 and STE-U 4416; results not shown). This might indicate that isolates within species can be divided into different virulence groups. This corresponds with earlier findings of Larignon *et al.* (2001) that isolates of *B. obtusa* could be

divided into four virulence groups. The four species that were not found among the South African isolates, *B. dothidea*, *B. lutea*, *B. ribis* and *B. stevensii*, were tested in the *in vitro* mature cane trial only. *Botryosphaeria ribis* and *B. stevensii* should be considered as potentially important pathogens of grapevines, while data for *B. lutea* and *B. dothidea* suggest that they are less virulent species. The variability observed here is an indication that the protocols used for pathogenicity testing should be standardised and should employ inoculation techniques that simulate natural infection.

The multifaceted approach of using different data sets to identify cryptic species of *Botryosphaeria* is in contrast to the earlier, more simplistic view taken by von Arx and Müller (1954), where 183 taxa were reduced to a core 11 species. Although relatively easy to apply, this concept does not reflect the diverse species of *Botryosphaeria*, their relative pathogenicity, distribution and ecology. Furthermore, all published records since von Arx and Müller (1954) should be treated with caution. Regarding the *Fusicoccum* complex, progress has been made by the characterisation and distinction of *B. dothidea*, *B. parva* and *B. ribis* (Slippers *et al.*, 2004a). The problem of dealing with these old names is one that will remain with us for some time to come. Approximately 2000 anamorph names are currently linked to the *Botryosphaeria* complex, with treatments of other genera continually also adding more. For instance, in their recent revision of *Phyllosticta* Pers. (Van der Aa & Vanev, 2002), an additional 18 species were recombined into *Fusicoccum*. Given the fact that none of these are known from culture and that recent studies suggest that culture and sequence data are required to clearly elucidate species of *Botryosphaeria*, it seems impossible to resolve the status of the old names in this group.

Although some species of *Botryosphaeria* appear to be host-specific, such as *B. protearum* Denman & Crous on *Protea* spp. (Denman *et al.*, 2003). Others, such as *B. obtusa*, *B. parva*, *B. rhodina*, etc., appear to be common, having wider host ranges and distributions than initially accepted.

The delimitation of new species of *Fusicoccum* from the *B. ribis/parva* complex underlines the fact that these species will not be identifiable without molecular data. Their conidial shapes and dimensions show considerable overlap. The reference strains at CBS, and sequence data available in GenBank will facilitate future identifications. It does raise perplexing questions for quarantine officers who need to have rapid tools in order to make decisions regarding the import and export of plant material.

The present phylogeny (Fig. 1) supports the decision of Denman *et al.* (2000) and Zhou & Stanosz (2001) to place anamorphs of *Botryosphaeria* in either *Fusicoccum* (hyaline, thin-walled

conidia) or *Diplodia* (pigmented, thick-walled conidia). Anamorph genera such as *Botryodiplodia* (Sacc.) Sacc. and *Sphaeropsis* Sacc. should be treated under *Diplodia* Fr. *Diplodia porosum* has thick-walled conidia that are initially hyaline, eventually turning brown at maturity within the pycnidial locule. A rather unusual character is the fact that the conidial wall is covered with large pores. The latter phenomenon has been noted in *Diplodia pinea* (Desm.) J. Kickx f., which has pitted and smooth conidial types, that seems to correlate with different cryptic species in this complex (De Wet *et al.*, 2003). The pores on conidia of *D. porosum* are unusual, and distinct from the pits in the conidial wall of *D. pinea*. Furthermore, *D. porosum* clusters between *Diplodia* and *Fusicoccum*, and may represent a distinct anamorph genus. Pores appear to be phylogenetically more informative than the striations observed on the inner conidial surface wall of *Botryodiplodia theobromae* Pat. These different anamorphs are, however, all part of *Botryosphaeria*, which appears to be monophyletic.

Othia Nitschke ex Fuckel may be synonymous with *Botryosphaeria* (Booth, 1958; Laundon, 1973; Denman *et al.*, 2000). Booth (1958) obtained single ascospores of *Othia spiraeae*, and via cultural studies linked this teleomorph to *D. sarmentorum*, a fungus regarded as cosmopolitan with a wide distribution (Wollenweber, 1941). He also designated *O. spiraeae* as lectotype of the genus *Othia*. Our sequence data (Fig. 1), confirm Booth's observations relating *D. sarmentorum* (CBS 120.43) to his strain of *O. spiraeae* (IMI 063581b). The grapevine isolates appear to be phylogenetically distinct, suggesting that they probably represent one of the 145 taxa regarded as synonyms of *D. sarmentorum* by Wollenweber (1941). An examination of the type specimen of *D. viticola* Desm. (PC), revealed that it is in fact a synonym of *Diplodia mutila* Fr. & Mont. (A.J.L. Phillips, pers. comm.), and thus unavailable for our isolates.

Laundon (1973) and Denman *et al.* (2000) considered *Othia* a probable synonym of *Botryosphaeria*, suggesting that ascospore septation is not useful at the generic level. The latter feature has recently been rejected in separating *Sphaerulina* Sacc. (3-septate ascospores) from *Mycosphaerella* Johanson (1-septate ascospores) (Crous *et al.*, 2003). The denominator common between species of *Mycosphaerella* and those of *Sphaerulina* that were shown to belong to *Mycosphaerella*, was the morphology of their anamorphs. Similarly, this also appears to be the case for *Othia*, as a culture identified as *O. spiraeae*, is shown here to belong to *Botryosphaeria*. The final synonymy of *Othia* (1870) under *Botryosphaeria* (1863) as suggested by Denman *et al.* (2000), however, awaits type studies and fresh collections from which single ascospore isolates can be obtained. Given the plasticity of the current generic concept of *Botryosphaeria* and its anamorphs, therefore, we have chosen to describe *D. porosum* with its characteristic

pored conidial wall in *Diplodia*, thus maintaining two anamorph genera for *Botryosphaeria*, namely *Diplodia* and *Fusicoccum*.

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Table 1. Isolates subjected to DNA sequence analyses

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1α
STE-U 4599	<i>Botryosphaeria australis</i>	<i>Vitis vinifera</i>	R.S.A.	F. Halleen	AY 343408	
STE-U 4428	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343391	
STE-U 4596	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343405	
STE-U 4585	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343402	
STE-U 4590	<i>B. australis</i>	<i>Robinia pseudoacacia</i>	Portugal	A.J.L. Phillips	AY 343403	
STE-U 4582	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343401	
STE-U 4591	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343404	
STE-U 4434	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343396	

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4433	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343395	
STE-U 4430	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343393	
STE-U 4529	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343399	
STE-U 4429	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343392	
STE-U 4528	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343398	
STE-U 4427	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343390	
STE-U 4435	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343397	
STE-U 4432	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343394	
STE-U 4415; CBS 112877	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343385	AY 343346

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4426	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343389	
STE-U 4425; CBS 112872	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343388	AY 343347
STE-U 5054	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343412	
STE-U 4535	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343400	
STE-U 5040	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343411	
STE-U 5033	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343409	
STE-U 5039	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343410	
STE-U 4597	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343406	

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4418	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343387	
STE-U 4598; CBS 110864	<i>B. australis</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343407	AY 343348
STE-U 5149; Cap 007	<i>B. dothidea</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY 343415	
STE-U 5045; CBS 110484	<i>B. dothidea</i>	<i>V. vinifera</i>	Argentina	G. Marta	AY 343414	AY 343350
STE-U 4595; Cap 086; CBS 110487 ²	<i>B. dothidea</i> (= <i>B. populi</i>)	<i>Populus nigra</i>	Portugal	A.J.L. Phillips	AY 343413	AY 343349
STE-U 4593; Cap 002 ² ; CBS 112873	<i>B. lutea</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY 343417	AY 343352
STE-U 4594; Cap 037	<i>B. lutea</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY 343418	
STE-U 4592; Cap 058; CBS 110862	<i>B. lutea</i>	<i>Sophora japonica</i>	Portugal	A.J.L. Phillips	AY 343416	AY 343351

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4542;CBS 112876	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343434	AY 343353
STE-U 4539	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343432	
STE-U 4541	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343433	
STE-U 4436	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343419	
STE-U 4531	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343426	
STE-U 5037	<i>B. obtusa</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY 343446	
STE-U 5043	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343448	
STE-U 5034;CBS 112978	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343445	AY 343354

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 5031	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343443	
STE-U 5042	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343447	
STE-U 5032	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343444	
STE-U 4538	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343431	
STE-U 4537	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343430	
STE-U 4581	<i>B. obtusa</i>	<i>V. vinifera</i>	France	P. Laignon	AY 343439	
STE-U 4587	<i>B. obtusa</i>	<i>V. vinifera</i>	France	P. Laignon	AY 343441	
STE-U 5162	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343458	

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Table 1. Continued

Accession no. ¹	Species	Host	Country	GenBank no.		
				Collector	ITS	EF-1α
STE-U 5143	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343455	
STE-U 4532	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343427	
STE-U 4527	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343425	
STE-U 5147	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343456	
STE-U 4440	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343420	
STE-U 4442	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343422	
STE-U 4443	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343423	
STE-U 4545	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343437	

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4543	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343435	
STE-U 5052	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343449	
STE-U 5053	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343450	
STE-U 5161	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343457	
STE-U 5163	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343459	
STE-U 4441	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343421	
STE-U 4544	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343436	
STE-U 4444	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343424	

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4536	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343429	
STE-U 4546	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343438	
STE-U 5139	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343452	
STE-U 5140	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343453	
STE-U 5141	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343454	
STE-U 5129	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343451	
STE-U 5164	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343460	
STE-U 4586	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343440	

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1α
STE-U 4588	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343442	
STE-U 4533	<i>B. obtusa</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343428	
STE-U 4437;CBS 112931	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343466	AY 343358
STE-U 4439;CBS 113032	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343468	AY 343360
STE-U 4438;CBS 112930	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343467	AY 343359
STE-U 5253;CBS 110888	<i>B. parva</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY 343477	AY 343367
STE-U 4431	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343464	
STE-U 4530;CBS 110852	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343469	AY 343361

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 5049;CBS 112879	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343474	AY 343365
STE-U 4534	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F Halleen	AY343465	
STE-U 4417;CBS 112871	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343461	AY 343355
STE-U 4420;CBS 110867	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343462	AY 343356
STE-U 5130	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343475	
STE-U 5142;CBS 110871	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343476	AY 343366
STE-U 4424;CBS 112932	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343463	AY 343357
STE-U 5035	<i>B. parva</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY 343473	

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4584; CBS 110952	<i>B. parva</i>	<i>V. vinifera</i>	France	P. Larignon	AY 343471	AY 343363
CMW9077	<i>B. parva</i>	<i>Actinidia deliciosa</i>	New Zealand	S.R. Pennycook	AY236939	AY236884
CMW 9078	<i>B. parva</i>	<i>A. deliciosa</i>	New Zealand	S.R. Pennycook	AY236940	AY236885
CMW 9079	<i>B. parva</i>	<i>A. deliciosa</i>	New Zealand	S.R. Pennycook	AY236941	AY236886
CMW9080	<i>B. parva</i>	<i>Populus nigra</i>	New Zealand	G.J. Samuels	AY236942	AY236887
STE-U 4589; CBS 113050	<i>B. parva</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343472	AY 343364
STE-U 5051; CBS 110495	<i>B. rhodina</i>	<i>V. vinifera</i>	Argentina	M. Gatica	AY 343483	AY 343369
STE-U 4583	<i>B. rhodina</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343482	

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Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 4423	<i>B. rhodina</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343481	
STE-U 4421	<i>B. rhodina</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343479	
STE-U 4422	<i>B. rhodina</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343480	
STE-U 4419; CBS 112874	<i>B. rhodina</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343478	AY 343368
CMW7772	<i>B. ribis</i>	<i>Ribes</i> sp.	U.S.A.	B. Slippers & G. Hudler	AY236935	AY236877
CMW7773	<i>B. ribis</i>	<i>Ribes</i> sp.	U.S.A.	B. Slippers & G. Hudler	AY236936	AY236878
STE-U 5038; CBS 112875	<i>B. stevensii</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY 343484	AY 343370
STE-U 5046; CBS 110574	<i>Diplodia porosum</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343378	AY 343339

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1 α
STE-U 5132; CBS 110496 ²	<i>D. porosum</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343379	AY 343340
CBS 120.41	<i>D. sarmentorum</i>	<i>Prunus communis</i>	Norway	H.W. Wollenweber	AY 343377	AY 343338
STE-U 5131	<i>Diplodia</i> sp.	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343374	
STE-U 5048; CBS 112869	<i>Diplodia</i> sp.	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343373	AY 343336
STE-U 5148; CBS 112870	<i>Diplodia</i> sp.	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343376	AY 343337
STE-U 5146	<i>Diplodia</i> sp.	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343375	
STE-U 5041; CBS 112977	<i>Fusicoccum viticlavatum</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343380	AY 343341
STE-U 5044; CBS 112878 ²	<i>F. viticlavatum</i>	<i>V. vinifera</i>	R.S.A.	F. Halleen	AY 343381	AY 343342

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Country	Collector	GenBank no.	
					ITS	EF-1α
STE-U 5252; CBS 110887 ²	<i>F. vitifusiforme</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343383	AY 343343
STE-U 5050; CBS 110880	<i>F. vitifusiforme</i>	<i>V. vinifera</i>	R.S.A.	J.M. van Niekerk	AY 343382	AY 343344
IMI 063581b; CBS 113091	<i>Oothia spiraeae</i>	<i>Ulmus</i> sp.	U.K.	A. Sivanesan	AY 343384	AY 343345

¹ Acronyms of culture collections: STE-U – Department of Plant Pathology, University of Stellenbosch, South Africa; Cap – A.J.L. Phillips, Universidade Nova de Lisboa, Portugal; CMW – M.J. Wingfield, FABI, University of Pretoria, South Africa; IMI – CABI Bioscience, Egham, U.K.; CBS – Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.

² Ex-type cultures.

Table 2. Mean lesion length caused by *in vitro* inoculations with isolates of *Botryosphaeria* species on green shoots of the grapevine cultivar ‘Periquita’

Treatment	Mean lesion length (mm) ^a
<i>Botryosphaeria australis</i> (STE-U 4416, 4598, 5040)	65.29 a
<i>B. parva</i> (STE-U 5142, 4589, 4420)	57.30 a
<i>B. rhodina</i> (STE-U 5051, 4422, 4421)	17.22 b
<i>Fusicoccum vitifusiforme</i> (STE-U 5050, 5252)	10.74 b
<i>F. viticlavatum</i> (STE-U 5044, 5041)	10.36 b
<i>B. obtusa</i> (STE-U 4444, 4440, 5139)	7.97 b
<i>Diplodia</i> sp. (STE-U 5148, 5048, 5131)	4.86 b
<i>D. porosum</i> (STE-U 5046, 5132)	4.30 b
Agar plug	3.38 b
LSD (<i>P</i> = 0.05)	16.95

^a Means followed by the same letter are not significantly different.

Table 3. Mean lesion length caused by isolates of *Botryosphaeria* species following *in vitro* inoculations on mature canes of grapevine cultivars ‘Chardonnay’ and ‘Cabernet Sauvignon’

Treatment	Mean lesion length (mm) ^a
<i>Botryosphaeria ribis</i> (CMW 7773)	26.75 a
<i>B. australis</i> (STE-U 4598, 4425, 4415)	18.17 b
<i>B. stevensii</i> (STE-U 5038)	17.56 b
<i>B. parva</i> (STE-U 4589, 4420, 5253)	11.25 c
<i>Fusicoccum vitifusiforme</i> (STE-U 5252)	7.69 d
<i>Diplodia</i> sp. (STE-U 5048)	7.31 d
<i>B. dothidea</i> (STE-U 5045)	7.13 d
<i>B. obtusa</i> (STE-U 5034)	7.13 d
<i>F. viticlavatum</i> (STE-U 5044)	7.10 d
<i>D. porosum</i> (STE-U 5046)	6.38 de
<i>B. lutea</i> (STE-U 4593)	6.31 de
<i>B. rhodina</i> (STE-U 5051)	5.75 de
Agar plug	3.81 e
No treatment	3.81 e
LSD ($P = 0.05$)	2.922

^aMeans followed by the same letter are not significantly different.

Table 4. Mean lesion lengths in mature canes and mature wood (rotting and streaking) of grapevine cultivar 'Periquita', caused by in vivo inoculations with isolates of *Botryosphaeria* species

Treatment	Lesion length (mm) ^a		
	Mature- canes	Mature wood	
		Rotting	Streaking
<i>Botryosphaeria australis</i> (STE-U 4416, 4598, 5040)	12.89 ab	25.90 a	48.85 a
<i>Fusicoccum viticlavatum</i> (STE-U 5044, 5041)	7.32 c	12.13 bc	36.60 b
<i>F. vitifusiforme</i> (STE-U 5050, 5252)	7.73 c	11.35 bcd	32.72 b
<i>B. obtusa</i> (STE-U 4444, 4440, 5139)	8.08 c	13.54 b	29.44 bcd
<i>B. parva</i> (STE-U 5142, 4589, 4420)	15.01 a	10.53 bcd	24.71 cd
<i>Diplodia</i> sp. (STE-U 5148, 5048, 5131)	7.18 c	6.80 d	24.25 cd
<i>B. rhodina</i> (STE-U 5051, 4422, 4421)	11.73 b	12.64 bc	22.67 d
<i>D. porosum</i> (STE-U 5046, 5132)	13.22 ab	7.96 cd	22.01 d
Agar plug	4.18 d	6.72 d	6.72 e
LSD ($P = 0.05$)	2.99	4.99	8.96

^a Means followed by the same letter are not significantly different.

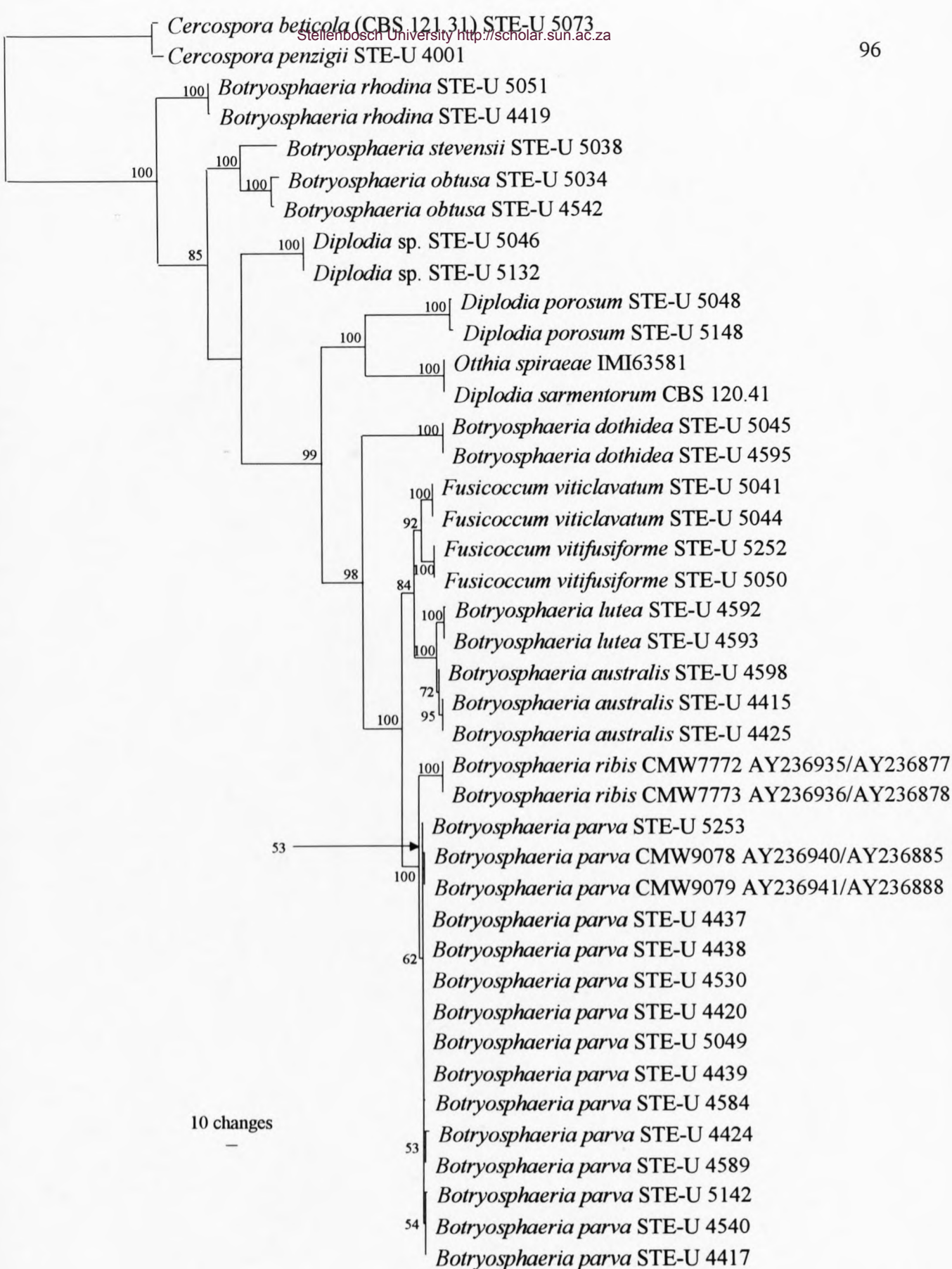


Figure 1. Single most parsimonious tree obtained from combined ITS and EF1- α sequence data. (PHT=0.220; TL = 1070 steps, CI = 0.742, RI = 0.888, RC = 0.659). Bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to *Cercospora beticola* STE-U 5073 and *Cercospora penzigii* STE-U 4001. The bar represents 10 changes.

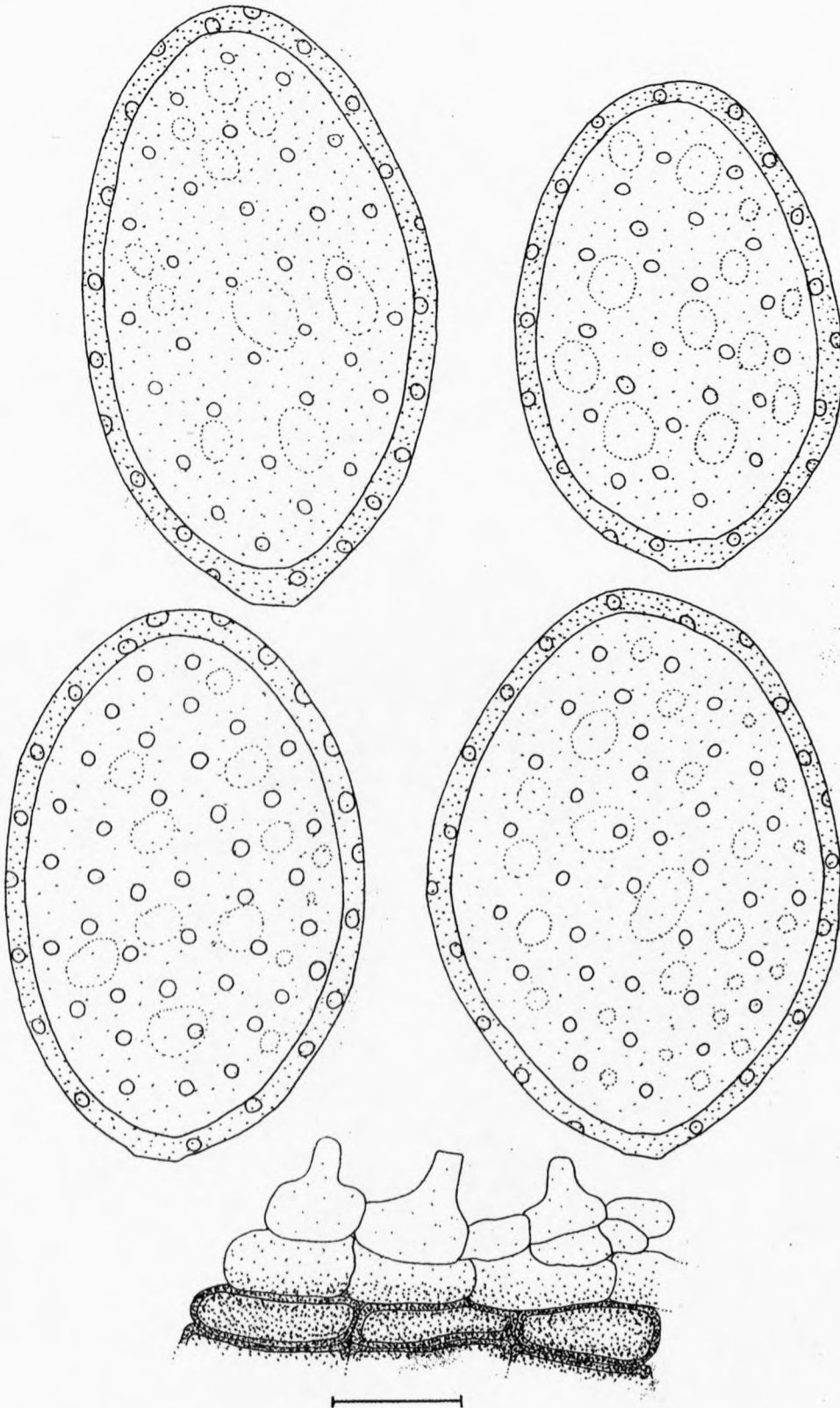


Figure 2. Conidia and conidiogenous cells of *Diplodia porosum* (holotype). Note pores on conidium body. Bar = 10 μ m.

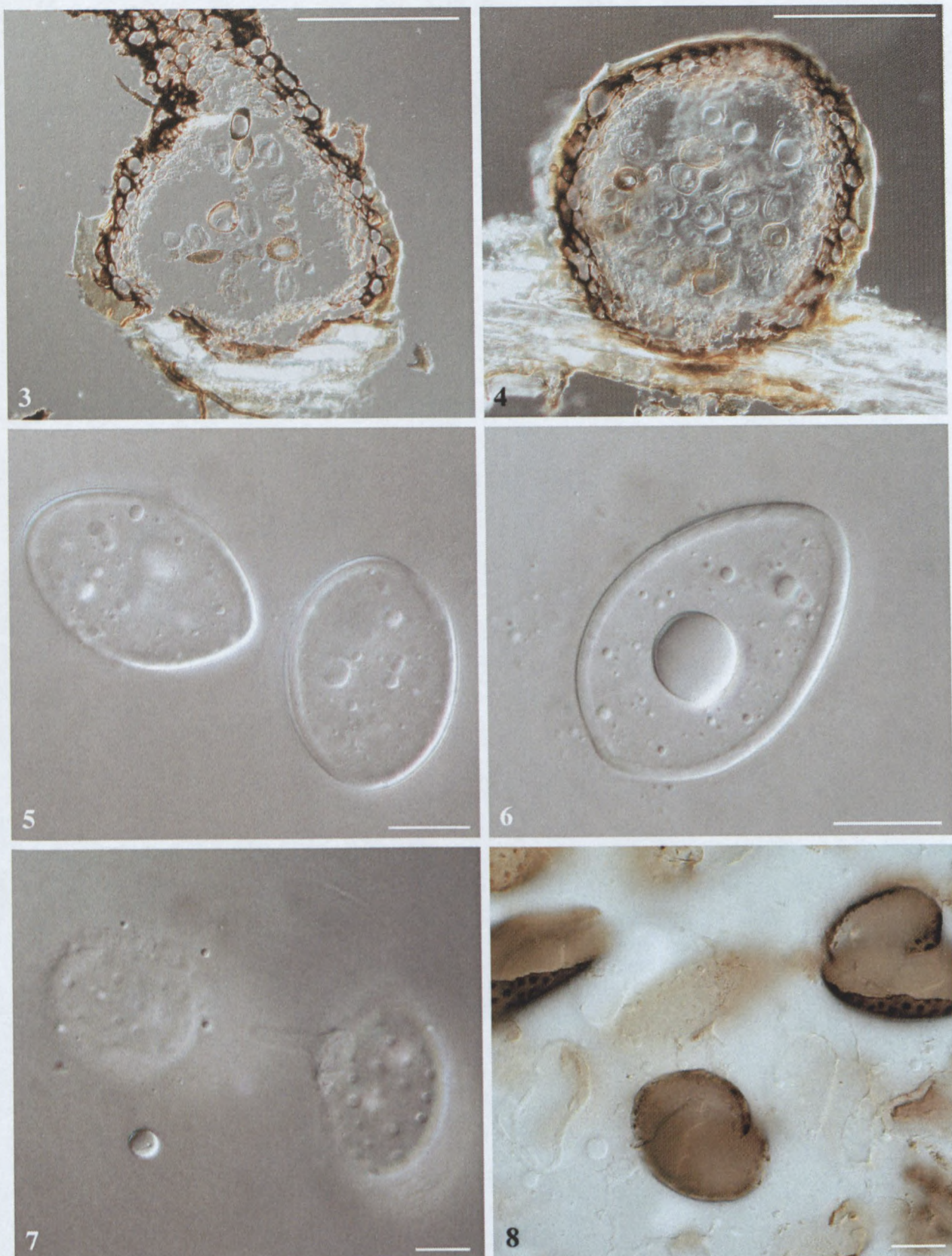


Figure 3–8. *Diplodia porosum* (holotype). **Figs. 3, 4.** Vertical section through pycnidia. **Figs. 5, 6.** Thick-walled conidia. **Fig. 7.** Pores visible on the conidial surface. **Fig. 8.** Broken, mature, pigmented conidia with pores. Bars = 200 μm in 3, 4; 10 μm in **Figs. 5–8.**

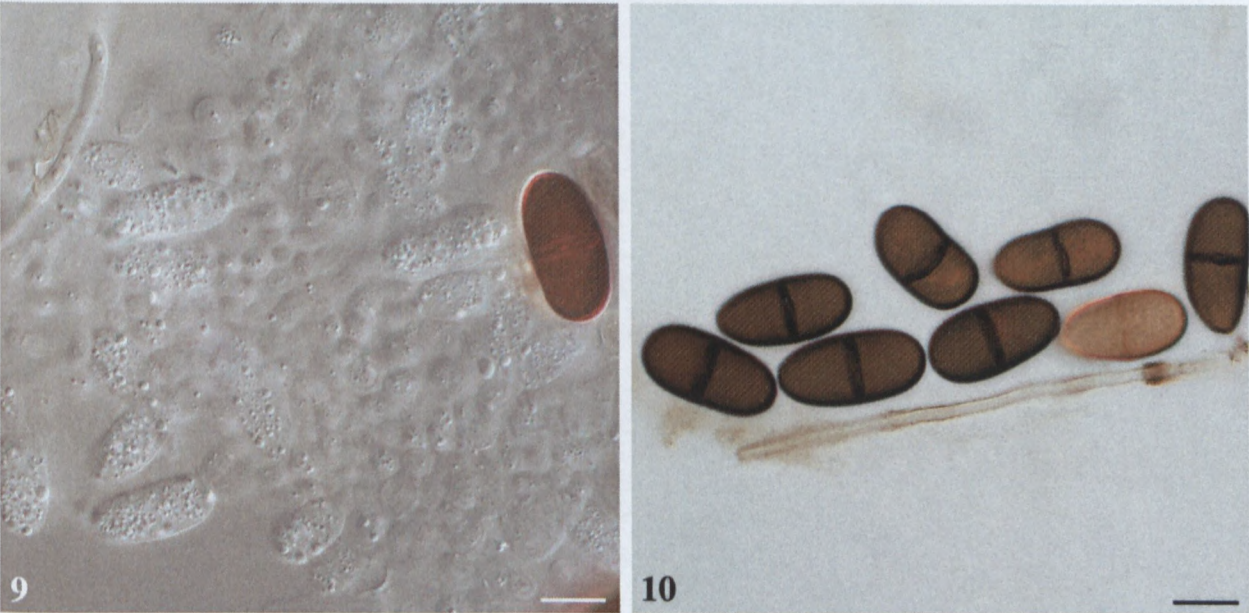


Figure 9, 10. Conidiogenous cells and conidia of a *Diplodia* sp. morphologically similar to *D. sarmentorum*. Bars = 10 μ m.

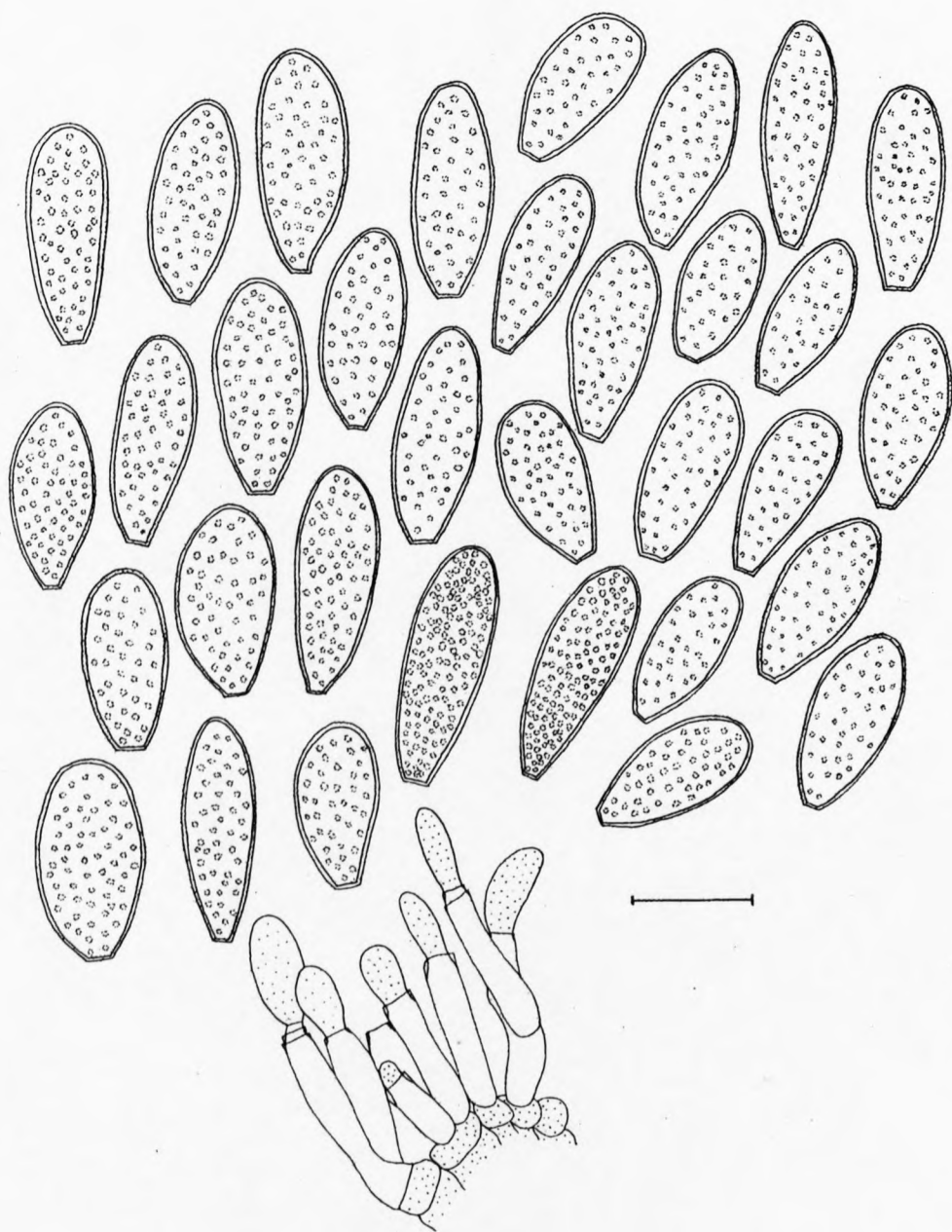


Figure 11. Conidia and conidiogenous cells of *Fusicoccum viticlavatum* (holotype). Bar = 10 μm .

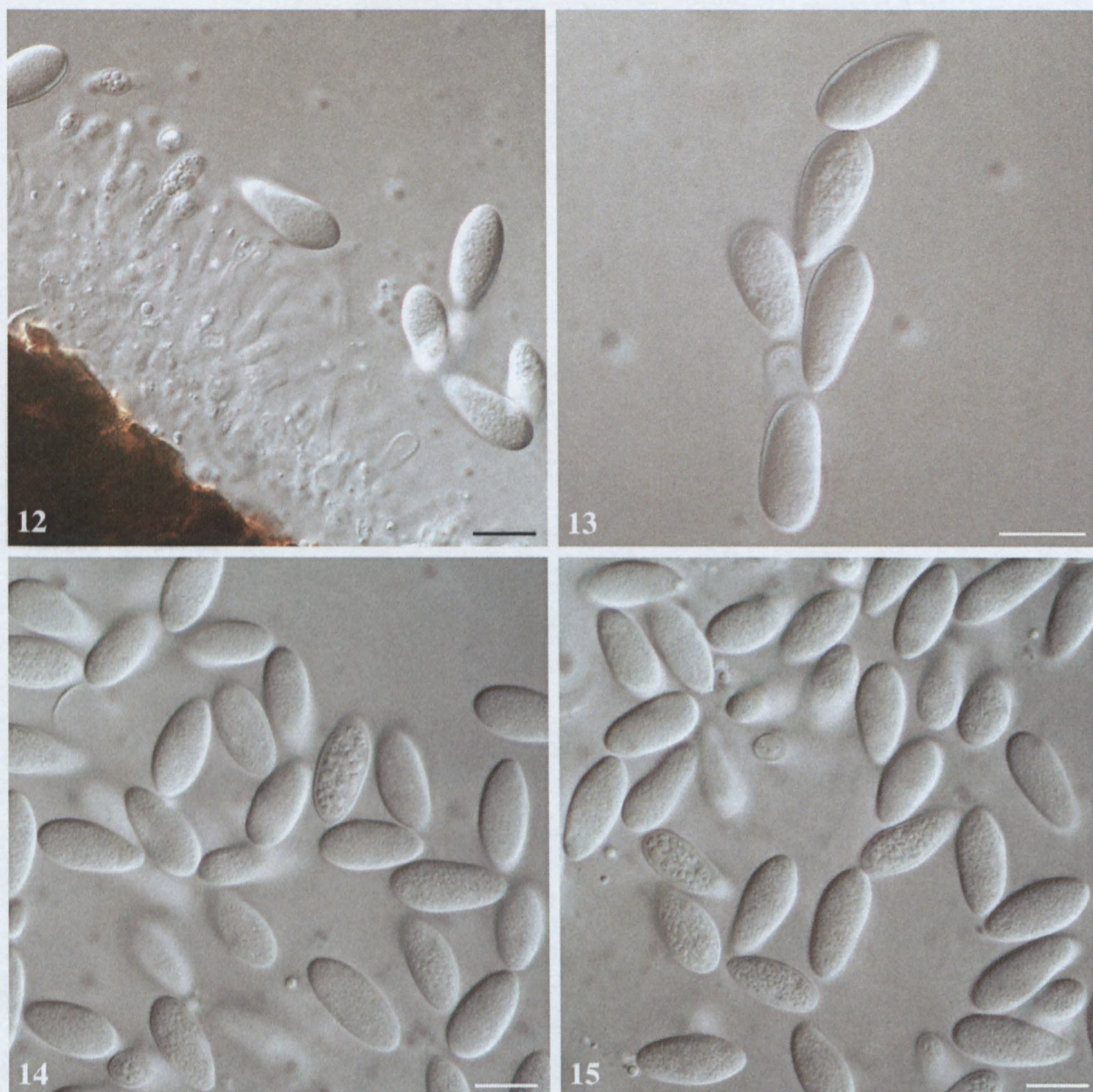


Figure 12–15. *Fusicoccum viticlavatum* (holotype). **Fig. 12.** Conidiogenous cells giving rise to conidia. **Figs. 13–15.** Conidia. Bar = 10 μ m.

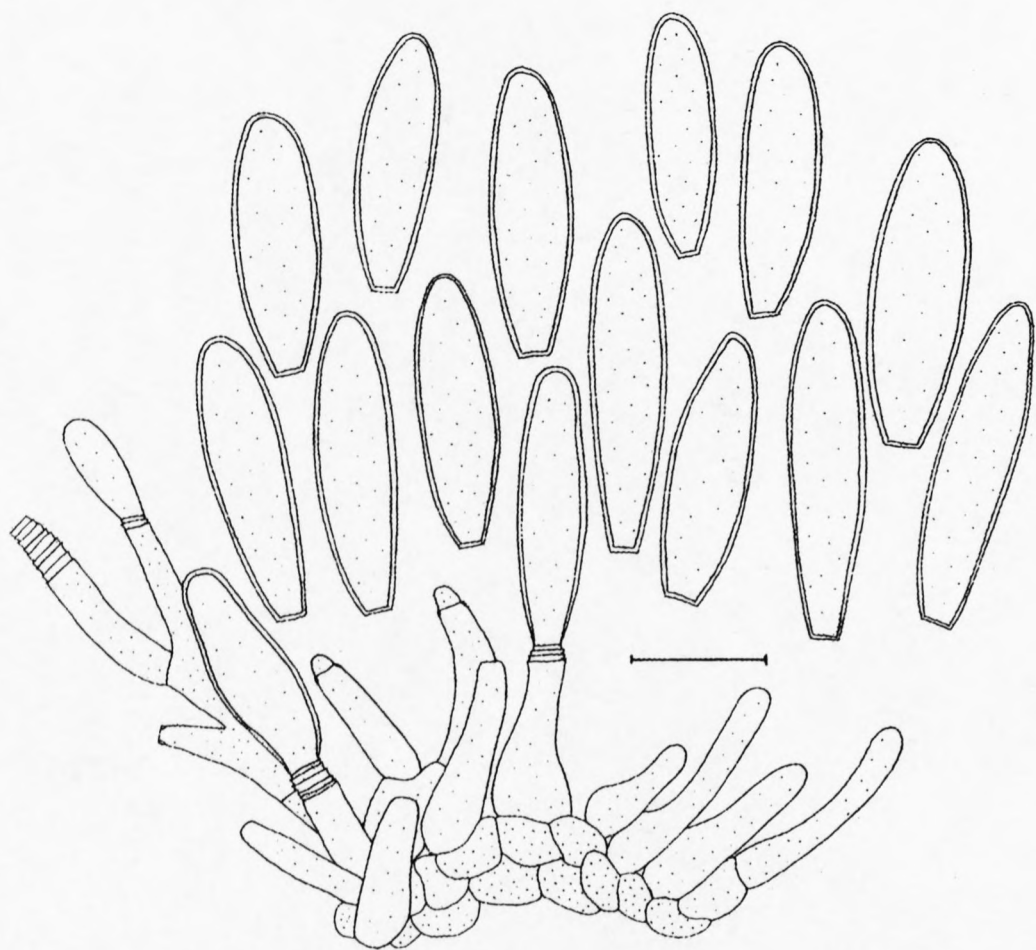


Figure 16. Conidia and conidiogenous cells of *Fusicoccum vitifusiforme* (holotype). Bar = 10 μ m.

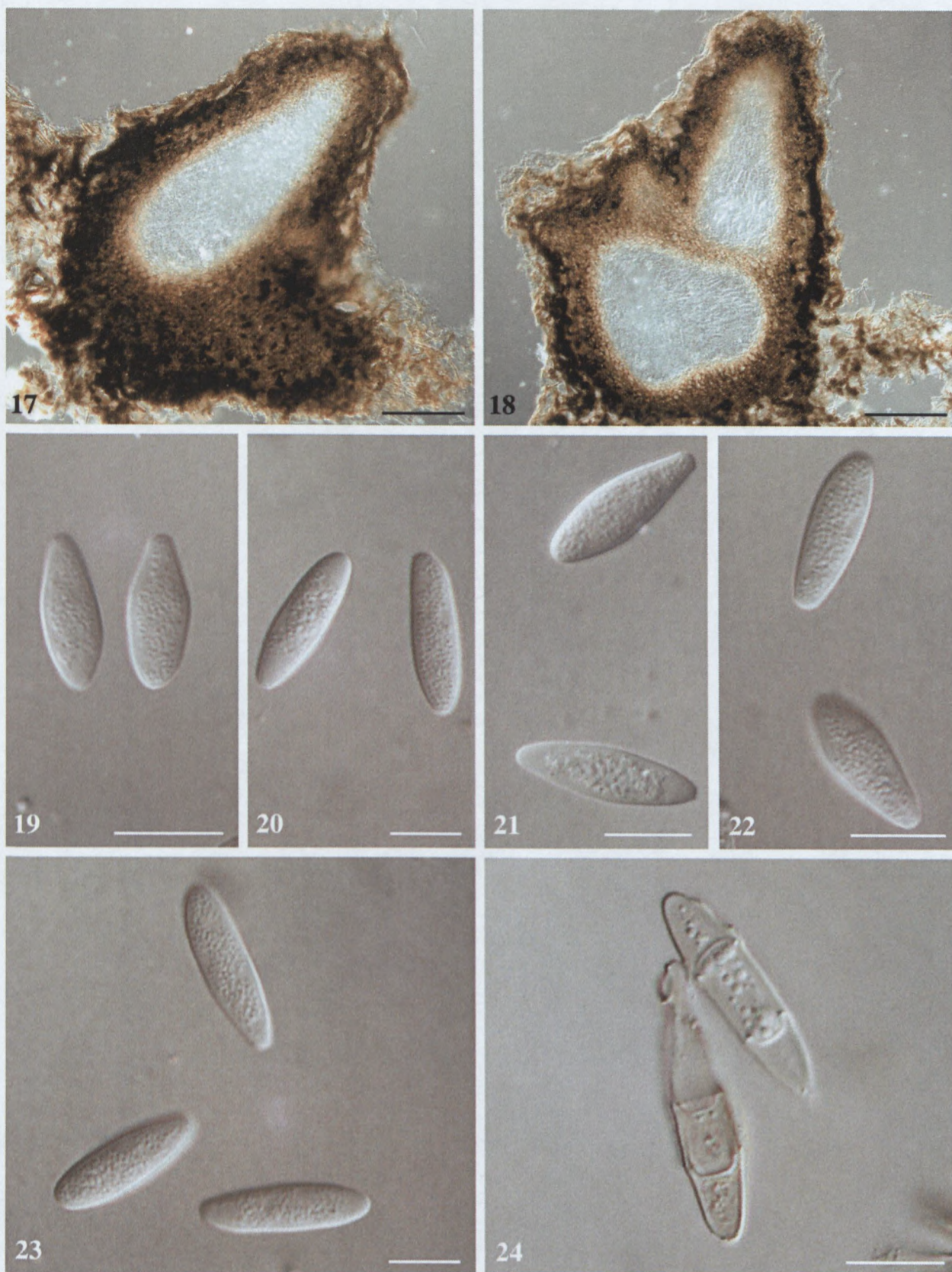


Figure 17–24. *Fusicoccum vitifusiforme* (holotype). **Figs. 17, 18.** Vertical section through pycnidia. **Figs. 19–23.** Mature conidia. **Fig. 24.** Conidia becoming 2-septate with age. Bars = 200 μm in 17, 18; 10 μm in Figs. 19–24.

4. REASSESSMENT OF *PHOMOPSIS* SPECIES ON GRAPEVINES

ABSTRACT

Ten species of *Phomopsis* have previously been identified from grapevines. Of these, *P. viticola*, the causal agent of Phomopsis cane and leaf spot, and *P. vitimegaspora*, causal agent of swelling arm of grapevines, have been confirmed as severe pathogens of this host. Earlier taxonomic treatments of *Phomopsis* species chiefly distinguished taxa based on host specificity, cultural characteristics and morphology. More recent studies have indicated, however, that these characteristics can no longer be used to distinguish species of *Phomopsis* due to the wide host ranges of some species, and the morphological plasticity of others. Using morphology, DNA sequences (ITS-1, 5.8S, ITS-2) and pathogenicity data, fifteen *Phomopsis* spp. were distinguished from grapevines in the present study. *Diaporthe helianthi*, a known pathogen of sunflowers, is for the first time reported from grapevines. A further six, presently unknown species of *Phomopsis*, are also distinguished from grapevines. A phylogenetic analysis of ITS data generated in this study distinguished three different clades containing isolates previously identified as *D. perijuncta*. Based on type studies, the name *D. viticola* can be applied to collections from Portugal and Germany. A new species, *D. australafricana*, is proposed for South African and Australian isolates formerly treated as *D. perijuncta* or *D. viticola*. A description for *D. perijuncta* is provided based on newly designated lectotype and epitype specimens. *D. perijuncta* is distinguished from *D. viticola* and *D. australafricana* based on morphology and DNA phylogeny. Artificial inoculations of green grapevine shoots indicated that, of the species tested, *P. amygdali*, a known pathogen of peaches in the USA, and *P. viticola* were the most virulent.

INTRODUCTION

The genus *Phomopsis* (Sacc.) Bubák contains more than 800 species that are recorded as being either plant pathogenic or saprobic (Uecker, 1988). Numerous species are routinely isolated from stems, roots, leaves and fruit of a wide variety of hosts (Rehner & Uecker, 1994). Species concepts in *Phomopsis* and its teleomorph *Diaporthe* Nitschke were initially based on morphological and cultural characteristics. However, due to the plasticity of these characteristics

(Rehner & Uecker, 1994), *Phomopsis* species concepts have in recent years mainly been based on host affiliation. Recent studies have shown, however, that various species of *Phomopsis* are able to infect a wide variety of hosts (Rehner & Uecker, 1994; Uddin *et al.*, 1997, 1998; Mostert *et al.*, 2001), and that host association is no longer sufficient for identification purposes. The use of anamorph/teleomorph relationships in identifying different *Phomopsis* species is also untenable, since *Diaporthe* teleomorphs have only been described for approximately 20% of the *Phomopsis* species known to date (Uecker, 1988).

Phomopsis cane and leaf spot is an important disease of grapevines, causing serious losses due to shoots breaking off at the base, stunting, dieback, loss of vigour, reduced bunch set and fruit rot (Pine, 1958, 1959; Pscheidt & Pearson, 1989; Pearson & Goheen, 1994). Ten different species of *Phomopsis* are currently known to occur on grapevines (Mostert *et al.*, 2001). Among these, only four have been confirmed as being pathogenic to this host, namely *P. viticola* (Sacc.) Sacc., *P. vitimegaspora* Kuo & Leu (teleomorph *Diaporthe kyushuensis* Kajitani & Kanem.), *P. amygdali* (Delacr.) J.J. Tuset & M.T. Portilla and a species previously referred to as *D. perjuncta* Niessl (Kuo & Leu, 1998; Kajitani & Kanematsu, 2000; Mostert *et al.*, 2001; Rawnsley *et al.*, 2001).

In an earlier study aimed at characterising the *Phomopsis* species occurring on grapevines, Merrin *et al.* (1995) employed morphological and cultural characteristics to distinguish four taxa within the *P. viticola* complex. Phillips (1999) concluded that the name *D. perjuncta* could be applied to isolates identified as taxon 1, while those treated as taxon 2 by Merrin *et al.* (1995) were representative of *P. viticola*. Confusion pertaining to the taxonomy of taxon 1 still remains, however, as Scheper *et al.* (2000) described taxon 1 isolates from Australia as *Diaporthe viticola* Nitschke. Although this species was initially regarded as the causal organism of grapevine bud mortality, it was later shown to only cause a bleaching of grapevine canes (Scheper *et al.*, 2000; Rawnsley *et al.*, 2001; Rawnsley & Wicks, 2002).

During the past two years a number of *Phomopsis* isolates were obtained from grapevines in the Western Cape province of South Africa. Isolations were made from severe *Phomopsis*-like symptoms, pruning wounds and asymptomatic nursery plants. Subsequent isolates obtained from this material varied greatly in morphology and cultural characteristics (pers. obs.). Given the wide host ranges and plasticity of morphological characteristics observed, it was difficult to identify species based on these characteristics (pers. obs.). The aims of this study were therefore to reassess the different *Phomopsis* species occurring on grapevines, using a combination of molecular, morphological, cultural, and pathological data, and to clarify the taxonomy of isolates previously identified as *D. perjuncta*.

MATERIALS AND METHODS

Isolates and morphology

Symptomatic and asymptomatic grapevine material and pruning debris were collected over a two-year period to obtain isolates of *Phomopsis* spp. Plant tissues were surface sterilised in 70% ethanol for 30 s, 1% NaOCl for 1 min, and again in 70% ethanol for 30 s before drying under a laminar flow hood. Small pieces of tissue were taken from the margin between necrotic and apparently healthy tissue, and plated out onto 2% potato dextrose agar (PDA; Biolab, Midrand, South Africa). The PDA plates were incubated at 25°C in the dark to promote mycelial growth. Single conidial isolations were made onto fresh PDA plates from sporulating colonies. In the case of the pruning debris, shoot pieces (5–7 cm long) were incubated in moist chambers in the laboratory for 1 week, and single spore colonies were made from sporulating pycnidia or perithecia. In order to induce sporulation, isolates were plated out on PDA, as well as water agar plates containing 4 cm long pieces of double autoclaved grapevine shoots (WAV). Inoculated plates were incubated at 25°C under nuv light in a 12 h light-darkness regime for 2–3 wks to enhance sporulation. To study the morphology of the teleomorph, isolates were plated on WAV, incubated for 3 wks at 25°C, and thereafter placed under nuv light at 10°C for a further 2 months. Fruiting structures were mounted in 70% lactic acid. Thirty measurements were taken of morphological structures, unless where otherwise stated, minimum and maximum ranges of spore dimensions recorded and averages determined. Growth rates, cultural characteristics and cardinal temperature requirements for growth were determined for isolates plated onto PDA in 90 mm Petri dishes and incubated in the dark for 7 days at seven different temperatures, ranging from 5°C to 35°C in 5°C intervals. Three plates were used for each isolate at each temperature. Radial mycelial growth was measured perpendicularly on each plate, and the mean calculated to determine the growth rate for each species. Culture colours were described from isolates incubated at 25°C for 7 days, using the colour scheme of Rayner (1970). Cultures are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U), and the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (CBS) (Table 1).

DNA isolation, amplification and sequence analysis

The isolation protocol of Lee and Taylor (1990) was used to extract genomic DNA from fungal mycelia grown on PDA. The primers ITS1 and ITS4 (White *et al.*, 1990) were used to amplify part of the nuclear rRNA operon spanning the 3' end of the 18S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5' end of the 28S (large subunit) of the rRNA gene. The PCR reaction mixture consisted of 1.5 units Biotaq (Bioline, London, UK), 1x PCR buffer, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 4 pmol of each primer, approximately 10 to 30 ng of fungal genomic DNA, and was made up to a total volume of 25 µl with sterile water. Reactions were performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) and the cycling conditions comprised of denaturation for 5 min at 96°C, followed by 30 cycles at 96°C (30 s), 55°C (30 s), 72°C (90 s) and a final 7 min extension step at 72°C to complete the reaction. PCR products were separated by electrophoresis in a 0.8% (w/v) agarose gel and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining. Amplification products were purified using NucleoSpin® Extract 2 in 1 kit (Macherey-Nagel, Germany).

Purified PCR products were sequenced in both directions using the PCR primers and the cycle sequencing reaction was carried out as recommended by the manufacturer with an ABI Prism Big Dye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA) containing AmpliTaq DNA Polymerase. The reaction was set up as follows: denaturing at 94°C for 5 min, followed by 25 cycles of 96°C for 10 s, 55°C for 10 s, and 60°C for 4 min, with a final incubation of 30 s at 60°C. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, CN).

The ITS nucleotide sequences generated in this study were added to other sequences obtained from GenBank (<http://www.ncbi.nlm.nih.gov>). *Valsa mali* Miyabe & G. Yamada (AF191186) and *Valsa japonica* Miyabe & Hemmi (AF191185) were included as outgroups. The alignments were assembled using Sequence Alignment Editor v2.0a11 (Rambaut, 2002) and manual adjustments for improvement were made where necessary. Phylogenetic analyses with neighbour joining (using uncorrected ("p") and Jukes-Cantor substitution models) were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2000). Alignment gaps were treated as missing data, all characters were unordered and of equal weight and any ties encountered were broken randomly. The robustness of the neighbour-joining tree was evaluated by 1000 bootstrap replications (Hillis & Bull, 1993).

Pathogenicity

Thirteen isolates, representing 12 different species of *Phomopsis* and a *Clonostachys rosea* (Link : Fr.) Schroers, Samuels, Seifert & W. Gams isolate, as the non-pathogen control, were selected as treatments. Other control treatments included were wounded only and inoculation with an uncolonised agar plug. The selected species were plated out on PDA and incubated at 25°C for 1 week. Inoculations were made on green shoots of the grapevine cvs 'Chenin Blanc' and 'Pinotage'. Shoot sections (internodes 2 to 4) were cut from vines *ca* 2 months after budburst and used for inoculations. Five shoot sections were used for each species. Shoot sections were wounded on internode 3 (2 mm deep) with a 3 mm cork-borer. A colonised agar plug, cut from a 1-week-old culture, was placed in the wound, which was subsequently covered with Parafilm. Inoculated shoots were incubated in the dark under moist conditions in the laboratory for 10 days at 22°C. Following the incubation period the external lesions on the shoots were measured. The layout of the trial was a completely randomised design and the data were statistically analysed using SAS (SAS, 1999). Analysis of variance and Student's t-tests for least significant differences were done. The experiment was done twice. After the experiment, all plant material was destroyed by autoclaving twice for 15 min.

RESULTS

Isolates and morphology

DNA was extracted from 63 isolates, which were isolated from symptomatic and asymptomatic material, and subjected to sequencing (Table 1). All isolates sporulated on PDA and WAV after being incubated at 25°C under nuv light in a 12 h light-darkness regime for 2–3 weeks. Teleomorphs were successfully induced for isolates of *D. ambigua* and *D. viticola* after 2 months incubation at 10°C under nuv light. Morphological descriptions and measurements are reported in the taxonomic part.

Sequence analysis

Approximately 510 to 530 bases were determined for each isolate and added to the alignment. The manually adjusted alignment (spanning ITS1, 5.8S rRNA gene, ITS2) of the nucleotide sequences contained 99 taxa and 510 characters including alignment gaps (data not

shown). Due to the inclusion of sequences from GenBank that were shorter on the 5' and 3' ends, the complete sequences determined in this study were not used in the phylogenetic analysis. Sequences were deposited in GenBank (Table 1), and the alignment in TreeBASE (SN 1693). The neighbour-joining analysis resulted in a phylogenetic tree delimiting six main clades and several sub-groups (Fig. 1). The topology of this tree was the same irrespective of which substitution model was used. The first main clade (85% bootstrap support) consists of *P. vitimegaspora* AF230749, an unknown species designated as *Phomopsis* sp. 3 (STE-U 4407), and two sub-groups. The first sub-group (bootstrap support of 99%) contains grapevine isolates that groups together with two GenBank sequences of *Diaporthe helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov. The second sub-group (bootstrap support of 100%) contains a single grapevine isolate, STE-U 5497, which groups together with sequences of *Diaporthe ambigua* Nitschke. The second major clade (68% bootstrap support) consists of four sub-groups. The first sub-group (97% bootstrap support) contains three grapevine isolates from Portugal, of which one (AF230765) was previously identified as *Diaporthe perijuncta* Niessl. The second sub-group contains additional isolates previously identified as *D. perijuncta* from South Africa (AF230744), and Australia (AF230760). These two sub-groups clusters with a bootstrap support value of 100%. The third sub-group in this clade (bootstrap support value of 100%) consists of three isolates of *Phomopsis amygdali* (Delacr.) J.J. Tuset & M.T. Portilla, two from grapevines in South Africa (STE-U 5151 and AF230755) and a GenBank sequence (AF102996). The fourth sub-group (76% bootstrap support) in this clade consists of five grapevine isolates (STE-U 5346, 5463, 5345, 5465, 5496), and represents an unknown taxon referred to as *Phomopsis* sp. 5. Another grapevine isolate, *Phomopsis* sp. 4 (STE-U 5464), clusters without any bootstrap support with the second main clade. The third main clade, *Phomopsis* sp. 6 (100% bootstrap support) contains several South African grapevine isolates that groups with a South African isolate from roses (AF230766), and another from cranberries in the USA (AF317580). The fourth main clade (68% bootstrap support) contains an isolate of *Phomopsis* sp. 2 (AF230761) and the ex-epitype strain of *D. perijuncta* (CBS 109745 = AR 3461). Included in this clade, is a sub-group containing isolates of *P. viticola* (100% bootstrap support). The fifth main clade (74% bootstrap support) contains *Phomopsis* sp. 7 (STE-U 5495) and a sub-group containing isolates of *Phomopsis* sp. 1. The *Phomopsis* sp. 1 subgroup (97% bootstrap support) consists of grapevine isolates from South Africa (STE-U 5567 and 5573, AF230743), Australia (AF230759, AF230750) and Portugal (AF230762). Additional South African isolates from *Protea* L. (AF230757) and *Pyrus* L. (AF230769) also clusters in this subgroup. The sixth main clade (100% bootstrap support) contains *Phomopsis* sp. 8 (STE-U 5462), which clustered with two unnamed *Phomopsis* species occurring on plum and pear trees in the USA.

Taxonomy

***Diaporthe viticola* Nitschke, *Pyrenomyces Germanici* 2: 264 (1870).**

Description. Phillips (1999), Mostert *et al.* (2001).

Cultures. Colonies convex with undulating, smooth margins, buff (19''f) on the surface, and rosy buff (13''f) underneath, obtaining a radius of 30 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 5°C, max. 30°C, opt. 25°C.

Host. *Vitis vinifera*.

Distribution. Germany, Portugal.

Specimens examined. Germany, Westfalen, Münster, bei der Wienburg, on *Vitis vinifera*, Feb. 1866, Nitschke, holotype in B. Portugal, Santo Tirso, Burgaes, on *Vitis vinifera*, 16 Feb. 1998, A.J.L. Phillips, EPITYPE specimen Herb. CBS 7950, ex-epitype culture STE-U 5683, CBS 113201. Italy, Sardiniae, on *V. vinifera*, holotype of *Diaporthe silvestris* Sacc. & Berl., PAD 228.

Notes. The fungus that Merrin *et al.* (1995) referred to as *Phomopsis* taxon 1 on grapevines, was later argued to be the same as *D. perijuncta* by Phillips (1999), while Scheper *et al.* (2000) again referred to it as *D. viticola*. In a subsequent study, Mostert *et al.* (2001) chose to follow the arguments of Phillips (1999), and used the name *D. perijuncta* for taxon 1. At that stage, however, Mostert *et al.* (2001) noted that minor morphological differences existed in perithecia and ascospores between Portuguese versus South African and Australian material. The molecular analysis, however, did not clearly separate these taxa. Since then, a specimen from the original location and host of *D. perijuncta* has been collected, which proved to be morphologically similar, but phylogenetically distinct. Furthermore, additional grapevine isolates have also been studied, which resolved the fact that the South African and Australian isolates represent a distinct taxon from the Portuguese isolates. A re-examination of the type of *D. viticola* furthermore revealed that this name could indeed be used for the European collections, having ascospores that were (9–)12–15 x 3.5–4(–4.5) µm, being widest in the middle of the apical cell, and frequently having terminal mucous caps, as observed in the Portuguese isolates. The South African and Australian isolates have ascospores that are widest at the median septum, and these collections are described as new below. The type specimen of *D. silvestris* Sacc. & Berl. in PAD was also examined (A.J.L. Phillips, pers. comm.), and in this collection ascospores also proved to be widest in the middle of the apical cell, and were 11–16 x 3.5–5 µm in size, thus being very similar to *D. viticola*, but still distinct from the South African and Australian material. If future studies would reveal *D. silvestris* to be synonymous with *D.*

viticola, the latter name, which is older, would retain priority. An epitype specimen (herb. CBS 7950) and ex-epitype culture (CBS 113201) is herewith selected to be used as future reference for the name *D. viticola*.

***Diaporthe australafricana* Crous & J.M. van Niekerk, sp. nov.**

Diaporthe viticolae similis, sed peritheciis solitariis, ostiolis sursum rubro-brunneis, sparse hyphis obtectis, ascosporis fusoides, (8–)11.5–13(–15) x (2–)3.5–3.5(–4) µm distincta.

Etymology. Referring to its known distribution in Australia and Africa.

Description and illustration. Mostert *et al.* (2001).

Host. *Vitis vinifera*.

Distribution. Australia, South Africa.

Specimen examined. South Africa, Western Cape province, Stellenbosch, on Riesling grapevines, Nov. 1997, L. Mostert, HOLOTYPE specimen PREM 56458, ex-type culture STE-U 2655, CBS 113487.

***Diaporthe perijuncta* Niessl, *Hedwigia* 17: 44 (1878).**

Figs. 2–9

[≡ *Diaporthe conjuncta* Niessl, *Hedwigia* 15: 153 (1876) non (Nees) Fuckel 1869].

= *Diaporthe saccardiana* Kunze ex Sacc., *Sylloge Fungorum* 1: 628 (1882).

≡ *Chorostate saccardiana* (Sacc.) Trav., *Flora Italica Cryptogama* 2: 206 (1906).

Stroma on surface as papillate pustules, 0.5–2 mm in longest dimension, to 0.7 mm deep, penetrating into wood, stroma prosenchymatous with embedded host tissue from both periderm and wood, little differentiation between entostroma and ectostroma, strongly delimited to sides and bottom by a dark brown to black layer of thick walled compact hyphae, layer 25–50 µm thick, dorsal zone absent, ostioles generally singly erumpent or in small groups, never united, often surrounded by a whitish disk, 3–10 perithecia per stroma; perithecia globose to somewhat flattened, 360–450 x 260–400 µm, wall of several layers of compact, rectangular cells, with walls somewhat thickened, brown in outer layers becoming hyaline in inner layers, 30–50 µm thick, necks 200–400 µm long, 100–170 µm wide at apex, wall pseudoparenchymatous, cells dark brown to black, thick-walled, ostiole periphysate; asci unitunicate, cylindric-clavate, with a refractive ring apparatus, mostly biseriate 48–74(–78) x 7.5–10 (avg. 62 x 8.5, n = 60);

ascospores hyaline, smooth, fusiform, ends obtuse, one-septate, often constricted at septum, guttulate with two large oil globules per cell, 11–15 x 3–4.5 (avg. 12.8 x 3.8, n = 76), gelatinous appendages present on both ends, in KOH appendages appear as short, cylindrical to flaring projections with an irregular end, 2–4 x 1.5 µm, in lactic acid appendages cylindrical with rounded ends 1.5–5 x 1–1.5 µm. Anamorph not observed.

Cultures. Colonies flat with smooth, undulating margins, white on the surface and straw coloured (21'd) underneath, obtaining a radius of 29 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 15°C, max. 30°C, opt. 25°C.

Hosts. On fallen branches of *Ulmus campestris* and *U. glabra*.

Distribution. Austria, Germany

Specimens examined. Austria, St. Margareten i. Ros., Schwarzaupt-Ostseite, Grid square 9452/4, on *Ulmus glabra*, 1 Jun 2000, *Walter Jaklitsch 1480* (BPI 748437, ex-epitype culture CBS 109745 (=AR 3461), herein designated EPITYPE of *Diaporthe perijuncta*). Germany, Stralsund, on *Ulmus campestris*, *Fischer* (Rabenhorst, *Fungi europaei exsiccati*, Series nov., no. 2325 issued in 1876. The specimen of this number was examined from BPI in the bound exsiccati and is herein designated LECTOTYPE of *Diaporthe perijuncta*); Germany, Saxony, Neckendorfer Thal near Islebiam, on *Ulmus campestris*, May, 1875, *W. Krieger* (BPI 616993 Johs. Kunze, *Fungi selecti exsiccati* 123, herein designated LECTOTYPE of *Diaporthe saccardianum*; BPI 619992; Germany, Saxony, Nossen, Dec. 1885, Jul 1886, Apr 1887, *W. Krieger* (BPI 616992 Krieger, *Fungi Saxonici* 632).

Notes. *Diaporthe perijuncta* is a distinctive species that is apparently restricted to species of *Ulmus* (Ulmaceae) in Austria and Germany. This species is unusual within *Diaporthe* in having scattered, solitary or clustered beaks emerging through bark with relatively small stroma evident as raised areas and relatively large, appendaged ascospores. The entostroma is well delimited with a black line that goes around the entire stroma. None of the specimens examined nor the culture showed evidence of an asexual state.

The name *Diaporthe perijuncta* Niessl was published to replace the later homonym *D. conjuncta* Niessl 1876 non (Nees) Fuckel 1869; both names are based on the same type specimen. The lectotype and epitype specimens agree in all aspects except for the size of the asci and ascospores. In the lectotype specimen the asci and ascospores were difficult to examine possibly due to previous handling of the specimen. The asci and ascospores in the lectotype were slightly larger than given above. In the lectotype specimen the asci are 57–75 x 8.5–12.5 (avg. 66.9 x 10.2, n = 12) and the ascospores: 12.5–18 x 3.5–5 (avg. 15.5 x 4.3, n = 29). The asci and

ascospores on the epitype agree with the protologue in Niessl (1876) and Wehmeyer (1933) and are considered correct for this species. Based on an examination of type and authentic specimens, the name *Diaporthe saccardiana* is confirmed as a synonym of *D. perijuncta* as previously noted by Wehmeyer (1933). This name was originally proposed on a herbarium label but lacked a description thus the name dates from the publication of a description in Saccardo (1882).

***Phomopsis* sp. 1**

Description. Mostert *et al.* (2001).

Notes. This species was treated in detail by Mostert *et al.* (2001). It is known from grapevines in Australia, Portugal and South Africa, and also occurs on proteas and pears in the latter country (Mostert *et al.*, 2001; Figs. 40, 41). Presently this species appears to have a wide host range, and it is probable that future studies will be able to link these cultures to an established name.

Hosts. *Protea* sp., *Pyrus* sp., *Vitis vinifera*.

Distribution. Australia, Portugal, South Africa.

***Phomopsis* sp. 2**

Host. *Vitis vinifera*.

Distribution. Italy.

Notes. This species was treated in detail by Mostert *et al.* (2001). Although it is morphologically distinct given the shape of its alpha conidia (Mostert *et al.*, 2001; Fig. 46), we decline to name it as new as it is presently known from only one collection.

***Phomopsis* sp. 3**

Fig. 10A

Cultures. Colonies flat with undulating, smooth margins, white on the surface, and straw coloured (21'd) underneath, obtaining a radius of 18 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 30°C, opt. 25°C.

Host. *Vitis vinifera*.

Distribution. South Africa.

Notes. This species is presently known from a single collection (STE-U 4407), and has ellipsoidal, non-guttulate alpha conidia, (5–)6–8(–9) x (2–)2.5–3 μm .

***Phomopsis* sp. 4**

Fig. 10B

Cultures. Colonies umbonate with smooth, even margins, hazel (17''i) on the surface, and isabelline (17''i) underneath, obtaining a radius of 18 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 35°C, opt. 30°C.

Host. *Vitis vinifera*.

Distribution. South Africa.

Notes. This species is known from a single collection from vines in South Africa (STE-U 5464). It is characterised by having fusoid–ellipsoidal alpha conidia that are multiguttulate, and taper from an obtusely rounded apex to a subtruncate base, (6–)7–9(–10) x 2–2.5(–3) μm , and straight, curved or hamate beta conidia, 25–30 x 1–1.5 μm .

***Phomopsis* sp. 5**

Fig. 10C

Cultures. Colonies appressed, convex with smooth, even margins, white on the surface, and white to olivaceous (21''m) underneath, obtaining a radius of 16 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 15°C, max. 35°C, opt. 30°C.

Host. *Vitis vinifera*.

Distribution. South Africa.

Notes. This species is known from several collections obtained from grapevines in South Africa (STE-U 5346, 5463, 5345, 5465, 5496). Alpha conidia are 6–8(–9) x 2(–2.5) μm , and have a characteristic taper, being widest in the middle or upper third, and tapering sharply to a truncate base, 0.5 μm wide.

***Phomopsis* sp. 6**

Fig. 10D

Cultures. Colonies flat with smooth, even margins, hazel (17''i) to buff (19''d) on the surface, and brown-vinaceous (5''m) to honey (19''b) coloured underneath, obtaining a radius

of 12 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 35°C, opt. 30°C.

Hosts. *Rosa* sp., *Vaccinium* sp., *Vitis vinifera*.

Distribution. South Africa, USA.

Notes. *Phomopsis* sp. 6 appears to be common on grapevines in South Africa (STE-U 5348, 5133, 5160, 5467, 5134, 5466, 5347, 5135, 5158, 5461), but also occurs on other hosts such as *Rosa* (South Africa), and *Vaccinium* (USA). This species has relatively large, fusoid–ellipsoidal alpha conidia, (6–)7–9(–10) x 2–2.5 µm, being slightly shorter than those of *P. viticola* (Mostert *et al.*, 2001).

***Phomopsis* sp. 7**

Fig. 10E

Cultures. Colonies appressed, convex with undulating margins, white on the surface, and buff (19''d) to white underneath, obtaining a radius of 31 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 5°C, max. 30°C, opt. 25°C.

Host. *Vitis vinifera*.

Distribution. South Africa.

Notes. This species is presently known from a single South African collection (STE-U 5495). Morphologically it is characterised by having slender, fusoid–ellipsoidal alpha conidia, (8–)9–10(–11) x 2–2.5(–3) µm, being slightly narrow than those of *P. viticola* (Mostert *et al.*, 2001).

***Phomopsis* sp. 8**

Fig. 10F

Cultures. Colonies convex with smooth margins, white on the surface, and olivaceous–buff (21'''d) underneath, obtaining a radius of 10 mm after 7 days at 25°C. Cardinal temperature requirements for growth: min. 10°C, max. 35°C, opt. 30°C.

Hosts. *Prunus* sp., *Pyrus* sp., *Vitis vinifera*.

Distribution. South Africa, USA.

Notes. Based on phylogeny obtained here, the grapevine isolate (STE-U 5462) clustered with a *Phomopsis* sp. that is regarded to be a serious pathogen of pears and plums in the USA (Uddin *et al.* 1998). Morphologically it had alpha conidia that were slender, fusoid to narrowly

ellipsoidal, with acutely rounded apices and subtruncate bases, (6–)7–9 x 2–2.5(–3) μm . Uddin (1998) described the alpha conidia of the plum and pear isolates as being fusiform, biguttulate to multiguttulate. The conidial measurements of the plum isolate were given as 7.5–15 x 1.25–3 μm , and those of the pear isolate as 6.5–12.5 x 2.5–4.5 μm . Although phylogenetically similar, the US collections thus seem to be morphologically distinct, and a detailed study would be required to resolve their status.

Other species that occurred on grapevines included *P. vitimegaspora* (STE-U 2675) (Kuo & Leu, 1998; Mostert *et al.*, 2001), and *Diaporthe helianthi* Munt.-Cvetk., Mihaljč. & M. Petrov (anamorph *P. helianthi* M. Muntañola-Cvetković & M. Petrov), known previously only from *Helianthus annuus* L. (Muntañola-Cvetković *et al.*, 1981). Two species that are commonly associated with fruit trees also occurred on vines, namely *D. ambigua* Nitschke (STE-U 2657, STE-U 3390, STE-U 5497) (Mostert *et al.* 2001; Moleleki *et al.* 2002), and *P. amygdali* (Delacr.) J.J. Tuset & M.T. Portilla (STE-U 2632, STE-U 5151) (Mostert *et al.*, 2001). Most of the isolates obtained in this study, however, proved to be representative of *P. viticola* (Table 1), the common agent of Phomopsis cane and leaf spot disease (Mostert *et al.*, 2001).

Pathogenicity

Analysis of variance for mean lesion lengths caused by selected *Phomopsis* isolates and controls on green shoots of cvs ‘Chenin Blanc’ and ‘Pinotage’ showed significant interaction between cultivar and treatment (Table 2), and the mean lesion lengths for the two cultivars were therefore not pooled (Table 3). This interaction was most probably due to *P. amygdali*, which caused lesions on ‘Pinotage’ that were significantly larger than those on ‘Chenin Blanc’ (60.6 mm and 44.0 mm, respectively). Conversely, *P. viticola* formed significantly larger lesions on ‘Chenin Blanc’ than on ‘Pinotage’ (39.9 mm and 34.1 mm, respectively). *Diaporthe viticola* formed lesions on ‘Chenin Blanc’ and ‘Pinotage’ that were significantly larger (12.7 mm and 10.5 mm, respectively) than the *G. roseum* control. (6.2 mm and 7.3 mm, respectively) and more than twice the size of the agar plug (3.4 mm and 3.9 mm, respectively) and wounded-only controls (3.4 mm and 4.7 mm, respectively). A number of species, *D. ambigua*, *Phomopsis* sp. 1, *Phomopsis* sp. 3, *Phomopsis* sp. 4, *Phomopsis* sp. 6, *Phomopsis* sp. 7 and *Phomopsis* sp. 8, formed lesions on both grapevine cultivars that were not significantly larger (6.3 to 9.6 mm) than the *C. rosea* control, and in some cases not significantly larger than the agar treated and wounded-only controls. *D. helianthi* (4.1 mm and 5.4 mm) and *Phomopsis* sp. 5 (3.4 mm and 4.5 mm) formed lesions similar in size to that of the agar and wounded-only controls. Re-

isolations from the mature wood were successful and all species were identified as being the same as originally used in inoculations, thereby satisfying Koch's postulates.

DISCUSSION

In this study, *Phomopsis* isolates were obtained from typical *Phomopsis*-like symptoms, pruning wounds, pruning debris and asymptomatic nursery plants. Fifteen species of *Phomopsis* were found to occur on grapevines in the present study. Of these taxa, *P. viticola*, and *P. vitimegaspora* are currently known to be pathogens of grapevines, while six species of *Phomopsis*, and one species of *Diaporthe*, *Diaporthe viticola*, were shown to probably be minor pathogens of this host. A further two species, previously reported from other hosts, were newly reported from grapevines. These include *P. amygdali*, a known pathogen of peaches and almonds (Farr *et al.*, 1999), and *D. helianthi*, a known pathogen of sunflowers. These findings thus provide further support for host switching within *Phomopsis* (Mostert *et al.*, 2001). One of the unknown *Phomopsis* species also contains isolates obtained from grapevines, roses and cranberries. The possibility that a *Phomopsis* species could have more than one host has great implications for the management of diseases caused by *Phomopsis* species as alternate hosts might act as a source of inoculum. These findings furthermore support those of previous studies (Farr *et al.*, 1999; Mostert *et al.*, 2001) that host specificity cannot be used to distinguish between different *Phomopsis* species.

The *in vitro* pathogenicity trials tested the ability of mycelium of selected *Phomopsis* species to infect wounded green shoots of two grapevine cultivars. All species were successfully re-isolated from the respective lesions and should thus be considered as potential pathogens of grapevines. *Phomopsis amygdali* and *P. viticola* caused the most severe lesions on both cultivars tested. In this study, *P. amygdali* was isolated only once from asymptomatic nursery plants and together with the isolate from the study of Mostert *et al.* (2001) only two isolates from grapevines exists at this time. However, from the results of the pathogenicity trial it is clear that this species could potentially be a serious pathogen of especially green grapevine shoots. Apart from being isolated from typical *Phomopsis* cane and leaf spot symptoms, *P. viticola* was also isolated from pruning wounds. This indicates that *P. viticola* can infect pruning wounds. The other species identified in this study, showed variable degrees of lesion formation on the green shoots. These species were also isolated from pruning wounds or asymptomatic nursery plants. However, at this stage it remains unclear whether pruning wound infection by the various

Phomopsis species would lead to wood decay and whether asymptomatic infections in nursery plants would eventually cause disease in grapevines in vineyards.

The present study has also attempted to resolve the identity of isolates previously identified as *D. perijuncta*, *D. viticola*, or *Phomopsis* taxon 1 (Merrin *et al.*, 1995; Phillips, 1999; Scheper *et al.*, 2000; Mostert *et al.*, 2001). Based on their phylogeny, which supported minute morphological differences, three species could be distinguished. *Diaporthe perijuncta* was recollected from *Ulmus glabra* in Germany, and can be distinguished from *D. viticola* by the length-width ratio of the ascospores. In *D. perijuncta*, the length-width ratio of the ascospores is less than 3.6 while in *D. viticola* it is greater than 3.7. Among isolates on *Vitis* in Europe, ascospores were widest in the middle of the apical cell, frequently having polar mucous caps, correlating with the type specimen of *D. viticola*. In contrast, however, South African and Australian material had ascospores that were widest at the median septum, and which were phylogenetically distinct. A new species, *D. australafricana*, is proposed for collections from the Southern Hemisphere.

Distinguishing *Diaporthe perijuncta* on *Ulmus* from *D. viticola* and *D. australafricana* has additional implications, as several other pathogenicity tests have been conducted on isolates generally fitting the morphology of *D. viticola* (as *Phomopsis* taxon 1) (Rawnsley *et al.*, 2001; Melanson *et al.*, 2002), which led to certain conclusions about its pathogenicity and potential role as pathogen in vineyards. It is possible, therefore, that several groups were working with morphologically similar, but different *Phomopsis* species.

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Table 1. *Phomopsis* and *Diaporthe* isolates studied

Accession no. ¹	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 2657; CBS 114015	<i>D. ambigua</i>	<i>Prunus</i> sp.	Unknown	SA	S. Denman	AF 230767
STE-U 3390; CBS 112171	<i>D. ambigua</i>	<i>Prunus</i> sp.	Unknown	SA	A. Smit	AF 230768
STE-U 5497	<i>D. ambigua</i>	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485744
STE-U 2655 ² ; CBS 113487	<i>D. australaficana</i>	<i>V. vinifera</i>	Unknown	SA	L. Mostert	AF 230744
STE-U 2676; CBS 111886	<i>D. australaficana</i>	<i>V. vinifera</i>	Unknown	Australia	R.W.A. Scheper	AF 230760
	<i>D. helianthi</i>	<i>H. annuus</i>	Unknown	Italy	Unknown	AJ 312348
	<i>D. helianthi</i>	<i>H. annuus</i>	Unknown	Italy	Unknown	AJ 312366
STE-U 5355	<i>D. helianthi</i>	<i>V. vinifera</i>	Pruning debris	SA	J.M. van Niekerk	AY 485745
STE-U 5353	<i>D. helianthi</i>	<i>V. vinifera</i>	Pruning debris	SA	J.M. van Niekerk	AY 485746
STE-U 5344	<i>D. helianthi</i>	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485747
STE-U 5356	<i>D. helianthi</i>	<i>V. vinifera</i>	Pruning debris	SA	J.M. van Niekerk	AY 485748
STE-U 5354	<i>D. helianthi</i>	<i>V. vinifera</i>	Pruning debris	SA	J.M. van Niekerk	AY 485749
STE-U 5683; CBS 113201 ²	<i>D. viticola</i>	<i>V. vinifera</i>	Unknown	Portugal	A.J.L. Phillips	AY 485750
AR 3461 = CBS 109745 ²	<i>D. perijuncta</i>	<i>U. glabra</i>	Unknown	Austria	Unknown	AY 485785
STE-U 5685; CBS 113203	<i>D. viticola</i>	<i>V. vinifera</i>	Unknown	Portugal	A.J.L. Phillips	AY 485751
STE-U 2677; CBS 114011	<i>D. viticola</i>	<i>V. vinifera</i>	Unknown	Portugal	A.J.L. Phillips	AF 230765
STE-U 2632; CBS 111811	<i>P. amygdali</i>	<i>V. vinifera</i>	Bleached cane	SA	L. Mostert	AF 230755
	<i>P. amygdali</i>	<i>P. persica</i>		USA	W. Miller	AF102996
STE-U 5151	<i>P. amygdali</i>	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485752
STE-U 5578	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485753

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 5605	<i>P. viticola</i>	<i>V. vinifera</i>	Lesions on green shoots	SA	F. Halleen	AY 485754
STE-U 5157	<i>P. viticola</i>	<i>V. vinifera</i>	Lens-shaped lesions on dormant canes	SA	F. Halleen	AY 485755
STE-U 5576	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485756
STE-U 5604	<i>P. viticola</i>	<i>V. vinifera</i>	Lesions on green shoots	SA	F. Halleen	AY 485757
STE-U 5577	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485758
STE-U 5513	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485759
STE-U 2671; CBS 267.80	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	Italy	Unknown	AF 230747
STE-U 2672; CBS 323.77	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	Turkey	A. Anbavoylu	AF 230748
STE-U 5514	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485760
STE-U 5602	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485761
STE-U 2679; CBS 114010	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	USA	Unknown	AF 230745
STE-U 2673; CBS 111888	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	USA	Unknown	AF 230746
STE-U 2666; CBS 111880	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	Portugal	A.J.L. Phillips	AF 230758
STE-U 2662; CBS 114009	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	Australia	R.W.A. Scheper	AF 230763
STE-U 5570	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485762
STE-U 5639	<i>P. viticola</i>	<i>V. vinifera</i>	Lesions on green shoots	SA	F. Halleen	AY 485763
STE-U 5511	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485764
STE-U 2648; CBS 111876	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	SA	L. Mostert	AF 230753
STE-U 2638; CBS 114476	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	SA	L. Mostert	AF 230764

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 5509	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485765
STE-U 2646	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	SA	L. Mostert	AF 230752
STE-U 5637	<i>P. viticola</i>	<i>V. vinifera</i>	Lens-shaped lesions on bleached canes	SA	F. Halleen	AY 485766
STE-U 2642	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	SA	L. Mostert	AF 230756
STE-U 2669; CBS 111889	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	Portugal	A.J.L. Phillips	AF 230770
STE-U 5155	<i>P. viticola</i>	<i>V. vinifera</i>	Lens-shaped lesions on dormant canes	SA	F. Halleen	AY 485767
STE-U 2660 ² ; CBS 114016	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	France	P. Laignon	AF 230751
STE-U 2641; CBS 114478	<i>P. viticola</i>	<i>V. vinifera</i>	Unknown	SA	L. Mostert	AF 230754
STE-U 5572	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485768
STE-U 5510	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485769
STE-U 5512	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485770
STE-U 5638	<i>P. viticola</i>	<i>V. vinifera</i>	Bleached canes	SA	F. Halleen	AY 485771
STE-U 5579	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485772
STE-U 5568	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485773
STE-U 5571	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485774
STE-U 5603	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485775
STE-U 5599	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485776
STE-U 5569	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485777
STE-U 5565	<i>P. viticola</i>	<i>V. vinifera</i>	Lens-shaped lesion on dormant spur	SA	F. Halleen	AY 485778

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 5636	<i>P. viticola</i>	<i>V. vinifera</i>	Internal browning in bleached cane	SA	F. Halleen	AY 485779
STE-U 5575	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485780
STE-U 5600	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485781
STE-U 5566	<i>P. viticola</i>	<i>V. vinifera</i>	Lens-shaped lesion on dormant spur	SA	F. Halleen	AY 485782
STE-U 5574	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485783
STE-U 5601	<i>P. viticola</i>	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485784
STE-U 2675 ²	<i>P. vitimegaspora</i>	<i>V. vinifera</i>	Unknown	Taiwan	Unknown	AF 230749
STE-U 2656	<i>Phomopsis</i> sp. 1	<i>Pyrus</i> sp.	Unknown	SA	L. Basson	AF 230769
STE-U 5567; CBS 111884	<i>Phomopsis</i> sp. 1	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485723
STE-U 5573	<i>Phomopsis</i> sp. 1	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485724
STE-U 2661; CBS 114480	<i>Phomopsis</i> sp. 1	<i>V. vinifera</i>	Unknown	Australia	R.W.A. Scheper	AF 230759
STE-U 2664; CBS 111885	<i>Phomopsis</i> sp. 1	<i>V. vinifera</i>	Unknown	Australia	D.L. Whisson	AF 230750
STE-U 2668; CBS 111881	<i>Phomopsis</i> sp. 1	<i>V. vinifera</i>	Unknown	Portugal	A.J.L. Phillips	AF 230762
STE-U 2654; CBS 114014	<i>Phomopsis</i> sp. 1	<i>V. vinifera</i>	Unknown	SA	L. Mostert	AF 230743
STE-U 2659; CBS 114477	<i>Phomopsis</i> sp. 1	<i>Protea</i> sp.	Unknown	SA	S. Denman	AF 230757
STE-U 2674	<i>Phomopsis</i> sp. 2	<i>V. vinifera</i>	Unknown	Italy	Unknown	AF 230761
STE-U 4407; CBS 111887	<i>Phomopsis</i> sp. 3	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	G.J. van Collier	AY 485725
STE-U 5464; CBS 113193	<i>Phomopsis</i> sp. 4	<i>V. vinifera</i>	Pruning wound	SA	F. Halleen	AY 485726
STE-U 5345; CBS 114481	<i>Phomopsis</i> sp. 5	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485727

Continued

Table 1. Continued

Accession no. ¹	Species	Host	Symptoms	Origin	Collector	GenBank no.
STE-U 5346	<i>Phomopsis</i> sp. 5	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485728
STE-U 5463	<i>Phomopsis</i> sp. 5	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485729
STE-U 5465; CBS 114482	<i>Phomopsis</i> sp. 5	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485730
STE-U 5496	<i>Phomopsis</i> sp. 5	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485731
STE-U 5461; CBS 113194	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485732
STE-U 5467; CBS 113197	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485733
STE-U 5466	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485734
STE-U 2680	<i>Phomopsis</i> sp. 6	<i>Rosa</i> sp.	Unknown	SA	S. Denman	AF 230766
ATCC 56789	<i>Phomopsis</i> sp. 6	<i>V. macrocarpon</i>	Unknown	USA	D.M. Boone	AF 317580
STE-U 5135	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485735
STE-U 5158	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485736
STE-U 5160; CBS 110877	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485737
STE-U 5134	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485738
STE-U 5347	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485739
STE-U 5348	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485740
STE-U 5133; CBS 113196	<i>Phomopsis</i> sp. 6	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485741
STE-U 5495	<i>Phomopsis</i> sp. 7	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485742
STE-U 5462; CBS 113195	<i>Phomopsis</i> sp. 8	<i>V. vinifera</i>	Asymptomatic nursery plant	SA	F. Halleen	AY 485743

¹ STE-U = University of Stellenbosch culture collection, Stellenbosch, South Africa; CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Table 2. Analysis of variance of lesion length caused by *in vitro* inoculations with selected isolates of *Phomopsis* and controls on green shoots of the grapevine cultivars ‘Pinotage’ and ‘Chenin Blanc’

Source of variation	DF	SS	MS	SL
Model	38	51808.5	1363.4	< 0.0001
Repeat	1	92.2	92.2	0.0102
Shoots	8	206.7	25.8	0.0639
Treatment	14	50032.5	3573.8	< 0.0001
Cultivar	1	2.0	2.0	0.7037
Cultivar x Treatment	14	1475.1	105.4	< 0.0001
Error	255	3510.2	13.8	
Corrected total	293	55318.7		

Table 3. Mean lesion length caused by *in vitro* inoculations with selected isolates of *Phomopsis* and controls on green shoots of the grapevine cultivars ‘Pinotage’ and ‘Chenin Blanc’

Treatment	Mean lesion length (mm) ^a	
	Pinotage	Chenin Blanc
<i>P. amygdali</i> (STE-U 5151)	60.6 a	44.0 b
<i>P. viticola</i> (STE-U 5572)	35.1 d	39.9 c
<i>D. viticola</i> (STE-U 5683)	12.7 e	10.5 ef
<i>Phomopsis</i> sp. 4 (STE-U 5464)	8.4 f-i	9.6 e-g
<i>Phomopsis</i> sp. 6 (STE-U 5135)	6.2 h-m	8.6 f-h
<i>Phomopsis</i> sp. 8 (STE-U 5462)	7.7 f-j	8.6 f-h
<i>D. ambigua</i> (STE-U 5497)	6.2 h-m	8.1 f-i
<i>Phomopsis</i> sp. 7 (STE-U 5495)	6.4 g-m	7.6 f-j
<i>Phomopsis</i> sp. 1 (STE-U 5573)	5.2 i-m	6.8 g-l
<i>Phomopsis</i> sp. 3 (STE-U 4407)	6.3 g-m	6.6 g-m
<i>D. helianthi</i> (STE-U 5353)	4.1 k-m	5.4 h-m
<i>Phomopsis</i> sp. 5 (STE-U 5346)	3.4 m	4.5 j-m
Non-pathogen (<i>Clonostachys rosea.</i>)	6.2 h-m	7.3 f-k
Wounded only	3.4 m	4.7 j-m
Agar plug	3.4 m	3.9 lm
LSD (<i>P</i> = 0.05)	3.304	

^a Means followed by the same letter are not significantly different.

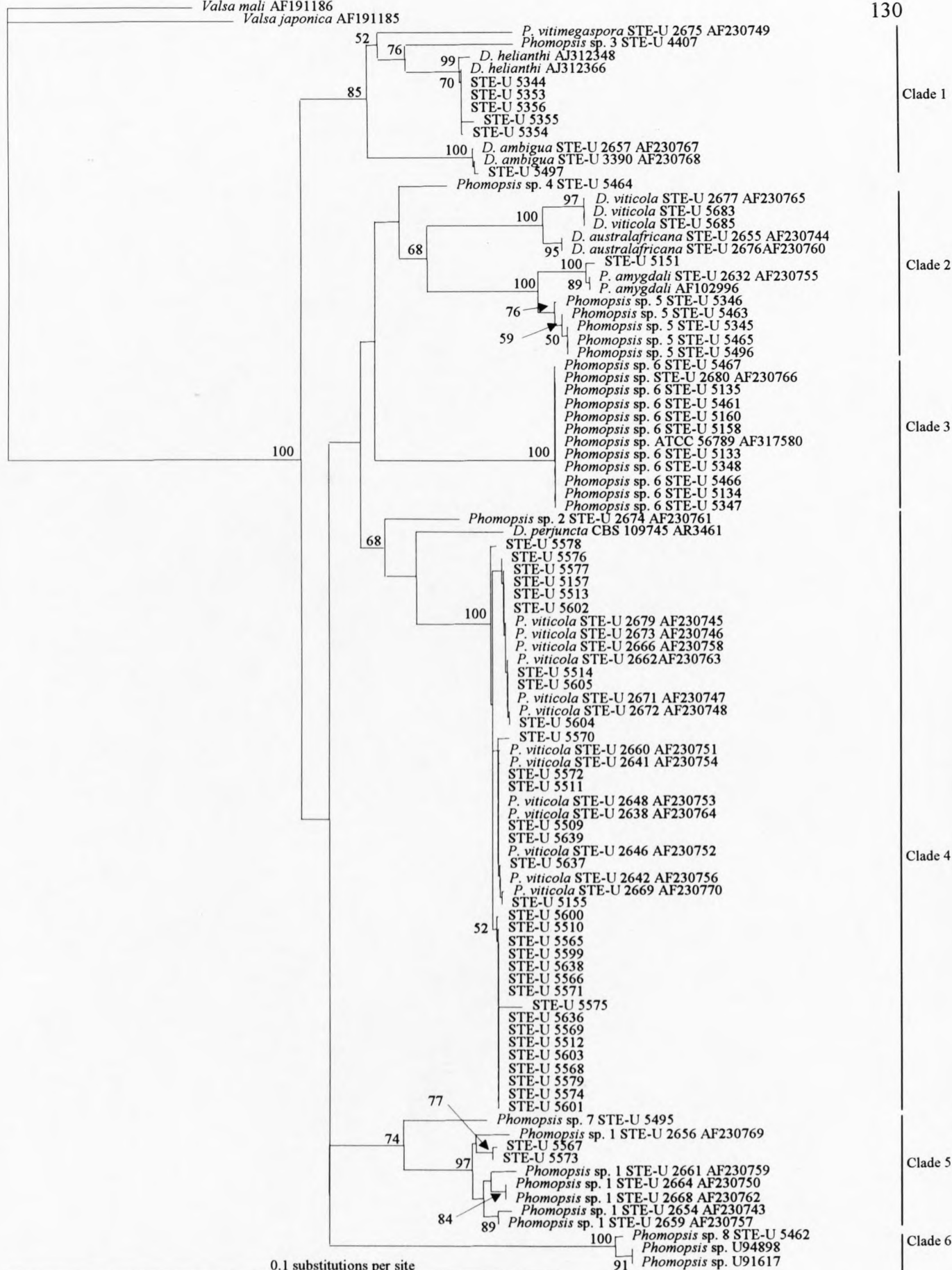
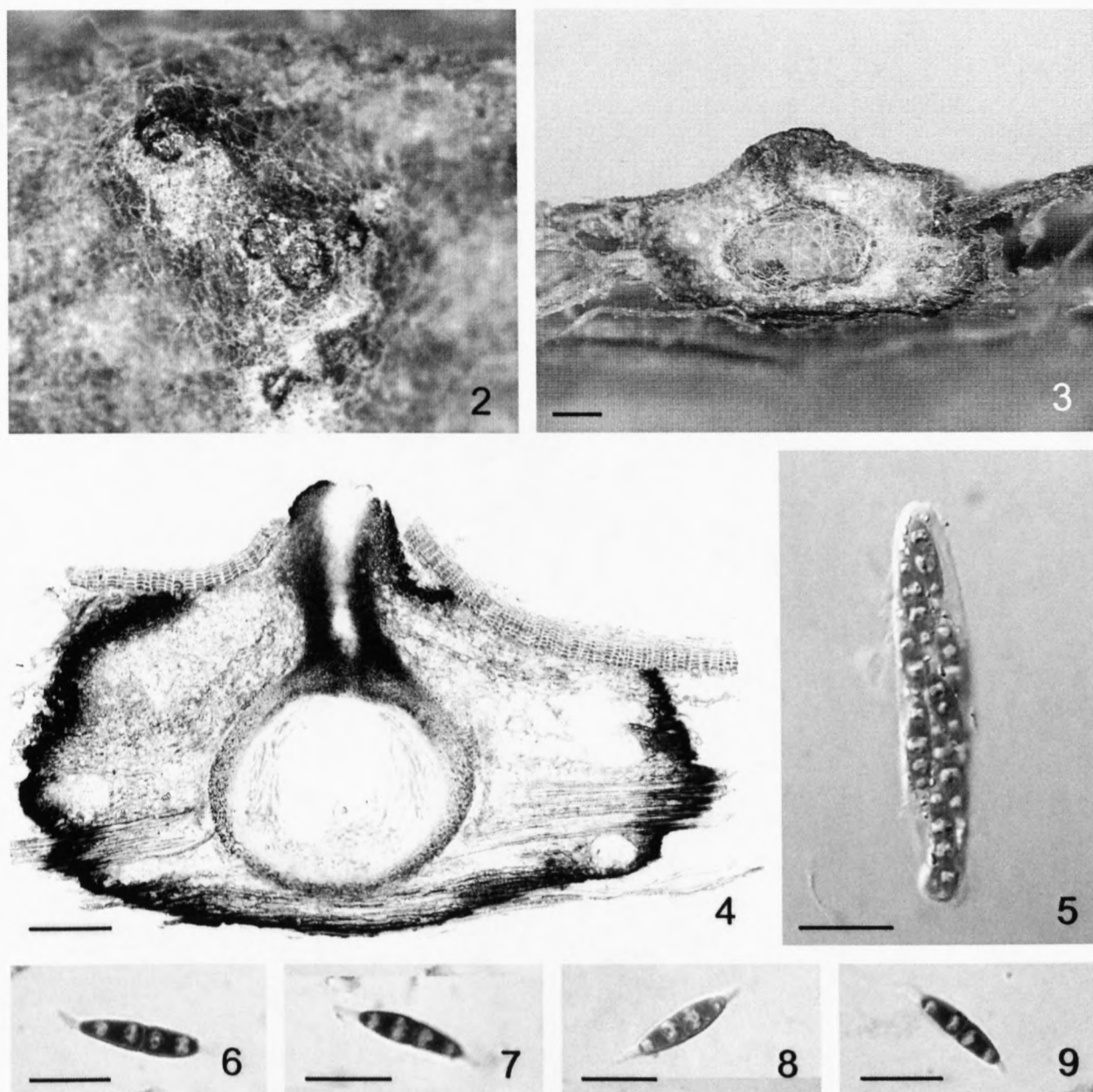


Figure 1. Neighbour joining tree of *Diaporthe* and *Phomopsis* spp. obtained from a phylogenetic analysis of aligned ITS sequence data. Bootstrap support values from a 1000 replicates are shown at the nodes. *Valsa mali* and *V. japonica* were used as outgroups. The scale bar shows the number of substitutions per site.



Figures 2–9. Morphological structures of *D. perijuncta*, (BPI 748437 = CBS 109745). **Fig. 2.** Stroma on host tissue. **Fig. 3.** Vertical section through stroma. Bar = 200 μ m. **Fig. 4.** Vertical section through a perithecium. Bar = 100 μ m. **Fig. 5.** Ascus with ascospores. Bar = 10 μ m. **Figs 6–9.** Ascospores with gelatinous appendages. Bar = 10 μ m.

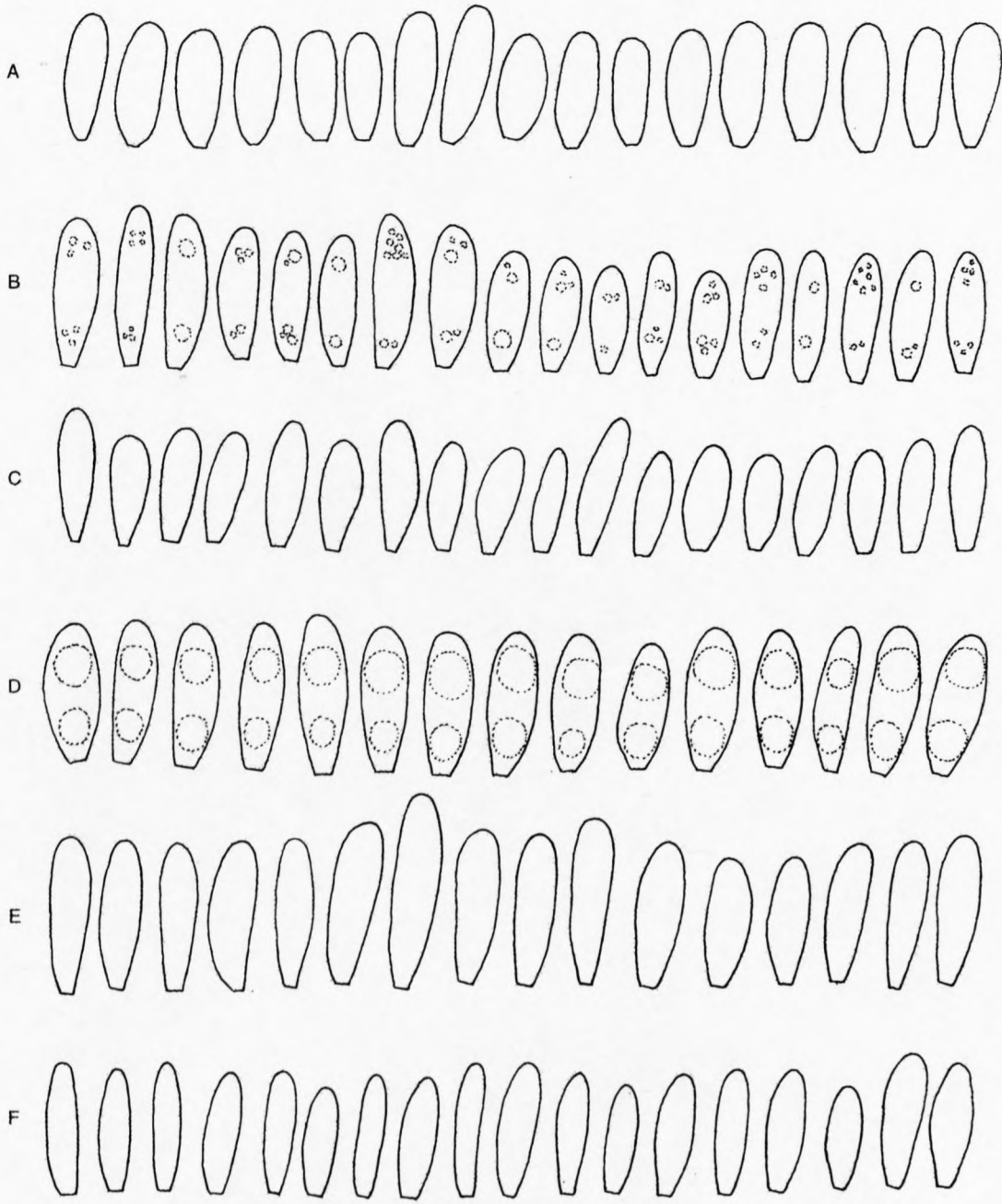


Figure 10A–F. Alpha conidia of various *Phomopsis* spp. as produced on grapevine canes *in vitro*. **Fig. 10A.** *Phomopsis* sp. 3 (STE-U 4407); **Fig. 10B.** *Phomopsis* sp. 4 (STE-U 5464); **Fig. 10C.** *Phomopsis* sp. 5 (STE-U 5496); **Fig. 10D.** *Phomopsis* sp. 6 (STE-U 5348); **Fig. 10E.** *Phomopsis* sp. 7 (STE-U 5495); **Fig. 10F.** *Phomopsis* sp. 8 (STE-U 5462). Scale bar = 10 μ m.